

Ascertainment of Phytochemical Screening, Antidiarrheal, Thrombolytic and Antibacterial effect of Methanol Extract of Leaves of *Zingiber rubens* Roxb.

Md. Abdul Barek*, A F M Shahid Ud-Daula, Md. Shafiullah Bhuiya, Md. Nazmul Huda, Md. Sohel Mia, Mohammad Anwarul Basher

Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.

Received: 28th Nov, 18; Revised 15th Mar, 19; Accepted 14th May, 19; Available Online: 25th Jun, 19

ABSTRACT

Zingiber rubens Roxb. (Family: Zingiberaceae) has been traditionally used against diarrhea, fever, cold, infection, etc. in human. The present study was aimed to investigate phytochemical screening, antidiarrheal, thrombolytic and antibacterial effect of methanol extract of leaves of *Z. rubens*. Phytochemical screening was performed according to common phytochemical tests. Antidiarrheal effect was evaluated by castor oil-induced diarrhea method at three different concentrations in mice. Five different concentrations were used for in vitro clot lysis assay of human blood and antibacterial activity was evaluated by disk diffusion assay at five different concentrations against two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive (*Micrococcus luteus* and *Staphylococcus aureus*) bacteria. Phytochemical screening showed presence of reducing sugars, anthraquinones, flavonoids, terpenoids, saponins, tannins, alkaloids and cardiac glycosides. In case of antidiarrheal activity, the extract asserted evacuation indices (EI) of 16.14, 15.57 ($p < 0.001$), 12.71 for 100, 200 and 400 mg/ml of extract respectively where loperamide showed 5.14 ($p < 0.001$). Percentage of clot disruptions were 47.21 ($p < 0.05$), 39.59 ($p < 0.05$), 35.31, 32.24 ($P < 0.01$), 24.88 ($p < 0.05$) and 55.93 ($p < 0.001$) for 10, 8, 6, 4, 2 mg/ml extract and streptokinase respectively. The extract did not possess any activity against bacteria. Based on the phytochemical and results from in vivo and in vitro activities, the leaves of *Z. rubens* was found to be a potential source of new antidiarrheal and thrombolytic agents but it was found to have no antibacterial activity.

Keywords: Phytochemical screening, anti-diarrheal, evacuation index, thrombolytic, antibacterial.

INTRODUCTION

In recent time, phytomedicines have drawn significant attention as therapeutic agents giving wide range of treatment options to diseases considering more beneficial than synthetic drugs. They are usually of low cost having fewer adverse effects and better efficacy in multidrug resistant outbreaks¹. Phytomedicines impart their therapeutic potentialities by a variety of compounds such as alkaloids, saponins, carbohydrates, glycosides, flavonoids, gums, steroids, terpenoids, phenolic compounds, volatile oils etc. extracted from numerous species of medicinal plants². Different parts of these medicinal plants are traditionally used as folk medicine in different geographic regions. Therefore, it is necessary to establish scientific evidences for therapeutic use of such medicinal plants. Zingiberaceae is a family of flowering plants made up of about 52 genera with a total of about 1600 known species³⁻⁶. The family is chiefly distributed throughout tropical Africa, China, Nepal, India, Bangladesh, Thailand, Indonesia, Malaysia, Singapore, Brunei, the Philippines and Papua New Guinea and the Americas⁵⁻⁷. *Zingiber rubens* Roxb. known locally as "Bengal Ginger", is a wild medicinal herb, which belongs to a family called Zingiberaceae. This plant is widely

found in Bangladesh, India, Myanmar, Thailand, Vietnam^{8,9}. Bengal Ginger leafy stems grow up to 6 ft. tall. Different parts of the plant have extensive uses in folk medicines for the treatment of various diseases such as diarrhea, fever, cough, cold, infection etc.^{10,11}. Another widespread species from same genus (*Zingiber*) in the same geographical region- *Zingiber officinale* Rosc. was reported to have antidiarrheal, thrombolytic and antibacterial effects¹²⁻¹⁵. Very few research works covering partial phytochemical and pharmacological studies of root and rhizome of *Zingiber rubens* Roxb. were reported^{16,17}, no phytochemical and pharmacological studies on leaves have been performed. This study deals with phytochemical screening and therapeutic evaluations as antidiarrheal, thrombolytic and antibacterial effects of leaves of *Zingiber rubens* Roxb.

MATERIALS AND METHODS

Drugs and chemicals

All chemicals and reagents used in this study were of analytical grade. Methanol (Merck, Germany) was used as a solvent during extraction. Sulphuric acid, chloroform, ammonia, ferric chloride, glacial acetic acid were excerpted from Merck, Germany. Standard streptokinase were

*Author for Correspondence: dr.mdbasher@gmail.com

Table 1: Phytochemical constituents of leave of *Zingiber rubens* Roxb.

Test	Leave of <i>Zingiber rubens</i> Roxb.
Reducing sugar	+
Anthraquinone	+
Terpenoids	+
Flavonoids	+
Saponins	+
Tannins	+
Alkaloids	+
Cardiac glycosides	+

‘+’ indicates presence of the component

collected from Popular Pharmaceuticals Limited, Bangladesh. Amikacin disc for antibacterial assay was procured from Becton, Dickinson and Company (Franklin Lakes, New Jersey, USA). Loperamide (Square Pharmaceuticals Limited, Bangladesh), castor oil (WELL’s Heath Care, Spain), olive oil (olitalia, Italy) and normal saline solution (0.9% NaCl, manufactured by Shazeb Pharmaceutical Industries, Bangladesh) were also used in this research.

Plant materials

Zingiber rubens was collected from rural region of Mowlobibazar, Sylhet district, Bangladesh on end of May 2017 and was identified by National Herbarium Institute, Mirpur, Dhaka, Bangladesh. (Accession number: DACB-44932).

Extraction

After collection of whole plants of *Z. rubens* was thoroughly washed with water. Then the selected plant part was dried and powdered. About 500 g of the powdered materials of plant was taken separately in a clean, flat bottomed glass container and soaked in 1500 ml of 80% methanol at room temperature for three weeks accompanying occasional shaking and stirring. Then the solution was filtered using filter cloth and Whatman filter paper (Bibby RE200, Sterilin Ltd., UK) and concentrated with a rotary evaporator (RE-EV311-V, LabTeck S.R.L, Italy). It rendered a gummy concentrate of greenish black color. The gummy concentrate was designated as crude methanol extract.

Experimental animals

All animal procedures and experimental protocols were approved by the Research Ethics Committee of the institution and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals¹⁸. Six-seven weeks old Swiss albino mice of both sexes with mean body weight 25 ± 5.0 g were procured from Jahangir Nagar University, Savar, Bangladesh. The animals were housed as 4 in 1 polycarbonate cage at a temperature (23 ± 1) °C and humidity (55-60%)-controlled room with a 12-h light-dark cycle. Animals were fed with a commercial rat pellet diet ad libitum during the entire experimental period. Ethical review board has approved to collect human blood.

Phytochemical screening

Phytochemical screening were performed using standard procedures described by Evans, 2009¹⁹ and Ayoola et al., 2008²⁰.

Test for reducing sugars (Fehling’s test)

0.5 g of methanol extract in 5 ml of water was added to boiling Fehling’s solution (A and B) in a test tube and was observed for change of colour.

Test for anthraquinones

0.5 g of extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was then shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of dilute ammonia was added. A rose pink to red colour indicated presence of anthraquinones.

Test for terpenoids (Salkowski’s test)

0.5 g of extract was added to 2 ml of chloroform. 3 ml of Concentrated sulphuric acid was added carefully and a reddish brown ring at the interfaces demonstrated the presence of terpenoids.

Test for flavonoids

5 ml of dilute ammonia was added to an aliquot of aqueous filtrate of extract followed by addition of 1 ml of concentrated sulphuric acid. The mixture turned yellow that disappears on standing indicating presence of flavonoids. Presence of flavonoids was further confirmed by addition of few drops of 1% aluminium solution to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids.

Test for saponins

0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. Then 3 drops of olive oil was added and again shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer’s reagent was added to one portion and Dragendorff’s reagent to the other. The formation of a cream (with Mayer’s reagent) or reddish brown precipitate (with Dragendorff’s reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)

0.5 g of extract was added to 5 ml in water followed by 2 ml of glacial acetic acid containing one drop of ferric chloride solution; then, 1 ml of concentrated sulphuric acid was added. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Effect on Castor oil induced diarrhea

The male Swiss mice’s were divided into five groups (n = 7). First group were orally treated with vehicle saline (10 ml/kg) and second group was orally treated with

Table 2: Effect of methanol extract of leaves of *Zingiber rubens* on diarrhea induced by castor oil in mice.

Treatment	Dose (mg/kg)	N	Initial Evacuation	Evacuation Classification			Evacuation Index (EI)	Inhibition ^a (%)
				Normal	Semi-solid	Liquid		
Vehicle	-	7	132.29 ± 10.77 ^{###}	1.71 ± .29	3.00 ± .31	5.53 ± .30 ^{###}	24.00 ± 1.09 ^{###}	-
Loperamide	5	7	290.86 ± 9.14 ^{***}	1.57 ± .90	1.14 ± .70	0.43 ± .43 ^{***}	5.14 ± 2.35 ^{***}	79
ME	100	7	156.14 ± 13.72 ^{###}	2.00 ± .65	2.71 ± .42	3.00 ± .49 ^{*#}	16.14 ± 2.46	33
	200	7	188.14 ± 24.32 [#]	4.43 ± .57 [*]	1.29 ± .52	2.85 ± .40 ^{**#}	15.57 ± 1.00 ^{***#}	35
	400	7	214.71 ± 22.70	2.29 ± .94	1.57 ± .61	2.42 ± .90	12.71 ± 3.30	47

Here, ME stands for methanol extract and Data are presented as mean ± S.E.M. ANOVA was employed, followed by Dunnett's test and significant differences were represented by *p<0.05, **p<0.01, ***p<0.001 vs control group treated with vehicle. For classification of evacuations and calculation of EI, Kruskal Wallis followed by Dunn. ^aInhibition in relation to evacuation index. [#]p<0.05 and ^{###}p<0.001 in relation to the loperamide.

loperamide (5 mg/kg). Third to fifth group were orally treated with methanol extract of leaves of *Z. rubens* at 100, 200 and 400 mg/kg accordingly. Castor oil (0.2 ml/animal) was administered after 30 minutes. Immediately after administering castor oil, each animal was kept in an individual cage with a floor lined with blotting paper and observed for 5 hours. The following parameters were monitored: time to initial evacuation, evacuation classification: 1 (normal stool), 2 (semi-solid stool), and 3 (watery stool) and evacuation index (EI). EI value was calculated according to the following formula: EI=1 x (no 1. stool)+2 x (no 2. stool)+3 x (no 3. stool)^{21,22}. Percentage of inhibition of diarrhea was calculated as (EI of vehicle -EI of sample) x 100/(EI of vehicle).

Thrombolytic activity

The thrombolytic activity of plant extracts was evaluated by the method developed by Prasad et al.²³ with modification to use streptokinase as standard^{24,25}.

Red blood cells (RBC) collection

Human RBCs were collected for conducting thrombolytic assay. Male volunteers- weighing average 65 and free from diseases were selected to collect RBCs (using a protocol approved by Institutional Ethics Committee).

Specimen

Five different concentrations were used to evaluate the thrombolytic activity of the plant extract. The extract of plants was dissolved in water and shaken vigorously on a vortex mixer to prepare different concentrations (2, 4, 6, 8 and 10 mg/ml respectively) of the test sample. The

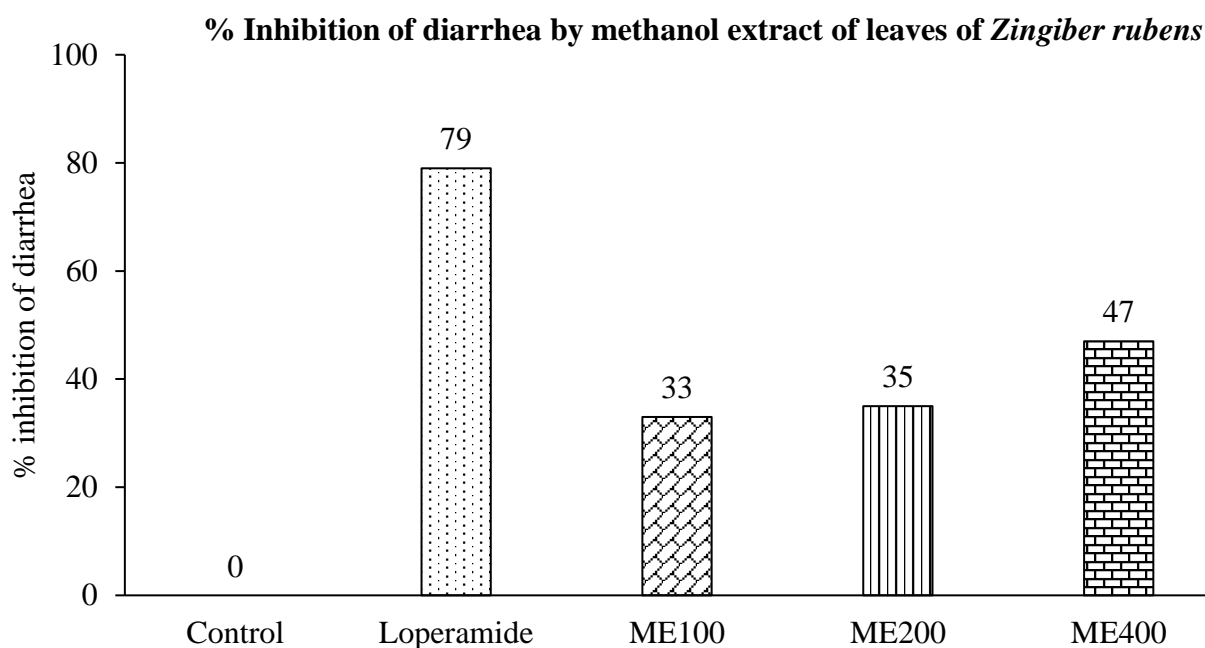


Figure 1: Effect of methanol extract of leaves of *Z. rubens* (100 mg/kg, 200 mg/kg and 400 mg/kg) with positive and negative control on % inhibition of diarrhea. % of inhibition were shown at the top of the bar of corresponding sample.

Table 3: Effects of different concentrations of methanol extract of leaves of *Z. rubens* on in-vitro clot lysis.

Treatment	Concentration	% of clot lysis for human blood (mean \pm SEM)
Water		1.20 \pm 0.60 ^{###}
Streptokinase	1,50,000 I.U.	55.93 \pm 0.93 ^{***}
ME	2 mg/ml	24.88 \pm 2.49 ^{*.#}
	4 mg/ml	32.24 \pm 1.30 ^{**.#}
	6 mg/ml	35.31 \pm 4.51
	8 mg/ml	39.59 \pm 2.60 [*]
	10 mg/ml	47.21 \pm 4.50 [*]

Here, ME stands for methanol extract and data was presented as mean \pm SEM. ANOVA was employed, followed by Dunnett's test and significant differences were represented by * p <0.05, ** p <0.01, *** p <0.001 vs control group treated with vehicle. Distilled water was employed as negative control and streptokinase was employed as positive control. # p <0.05, ## p <0.01 and ### p <0.001 in relation to the Streptokinase. For streptokinase, 100 μ l contained 1,50,000 I.U.

suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22-

micron syringe filter. 100 μ l of the plant extract with different concentrations were added to the eppendorf tube which contained the clots to check thrombolytic activity^{23,24}. Streptokinase was employed as positive control and water was employed as negative control.

Thrombolytic assay

During this study, 7 ml of venous blood was drawn from healthy volunteers (n = 3) and transferred to different pre-weighed sterilized eppendorf tube. The eppendorf tubes were incubated at 37 °C for 45 minutes. After formation of a clot, serum was completely discarded from the tubes (carried out without disturbing the clot formed). Each eppendorf tube was weighed to determine weight of the clot. Each eppendorf tube was appropriately labeled and 100 μ l of the plant extract with various concentrations (2, 4, 6, 8 and 10 mg/ml respectively) was added to the tubes accordingly. 100 μ l of streptokinase and 100 μ l of water were distinctly added to the control tubes numbered. The tubes were incubated again at 37 °C for 90 minutes and observed for clot lysis. After the following incubation, the obtained fluid was discarded from the tubes. They were again weighed to observe the weight of released clot^{23,24}. Every test samples were examined in triplicate. Finally, the result was expressed as percentage of clot lysis which is calculated by the following equation-

$$\% \text{ of clot lysis} = (\text{weight of released clot} / \text{clot weight}) \times 100\%$$

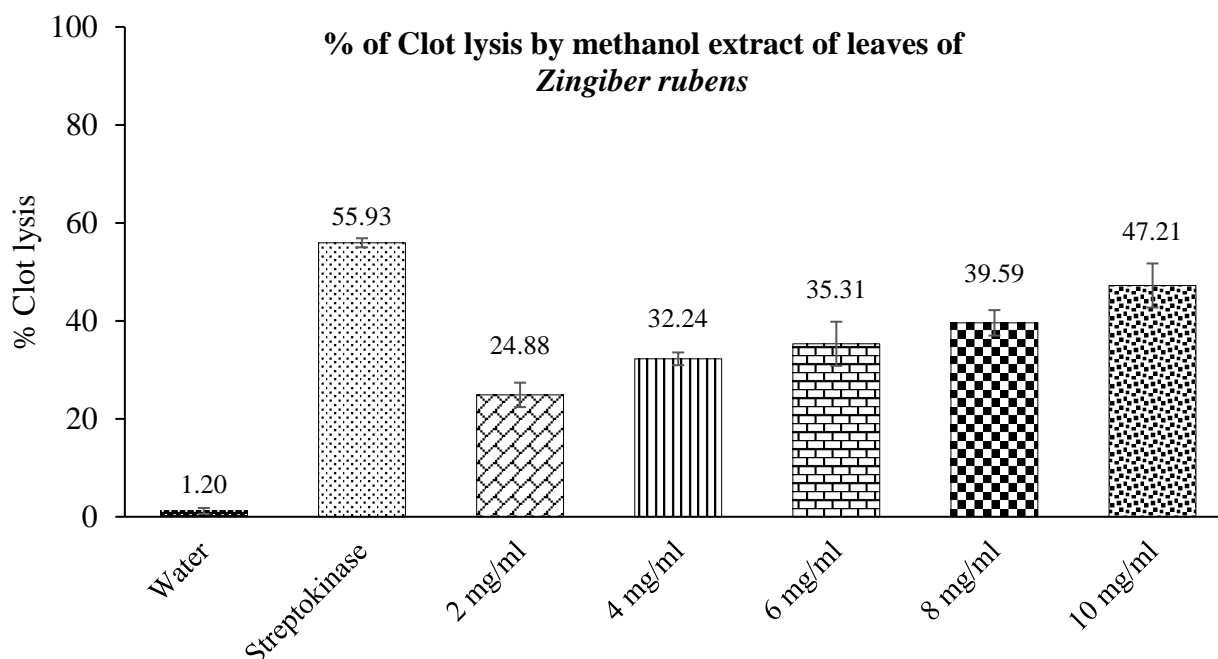


Figure 2: Effect of methanol extract of leaves of *Z. rubens* (2, 4, 6, 8 and 10; all concentrations were in mg/ml) with positive and negative control on % clot lysis on human blood. Water and Streptokinase were used as negative and positive control respectively. Values are expressed as Mean \pm SEM and all data were analysed using ANOVA followed by Dunnett's test and significant differences were represented by * p <0.05, ** p <0.01, *** p <0.001 vs control group treated with vehicle. Distilled water was employed as negative control and streptokinase was employed as positive control. # p <0.05, ## p <0.01 and ### p <0.001 in relation to the Streptokinase.

Table 4: Antibacterial activity of methanol extract of leaves of *Z. rubens*.

Microorganism	Mean inhibition zone (mm)					
	Standard (Amikacin)	Leaves extract				
	0.03 mg/disc	5 mg/disc	1 mg/disc	0.5 mg/disc	0.25 mg/disc	0.1 mg/disc
Gram Negative						
<i>Pseudomonas aeruginosa</i>	23 ± 00	-	-	-	-	-
<i>Escherichia coli</i>	23 ± 00	-	-	-	-	-
Gram Positive						
<i>Micrococcus luteus</i>	19 ± 00	-	-	-	-	-
<i>Staphylococcus aureus</i>	24 ± 00	-	-	-	-	-

‘-‘ indicates no effect; values are means ± S.E.M ($n = 3$)

Antibacterial assay

Microorganisms

A total of four different microorganisms were used to examine antibacterial potentials of methanol extract of leaves of *Zingiber rubens* leaves. Two species of Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 15422) and two species of Gram-positive bacteria (*Micrococcus luteus* ATCC 4698 and *Staphylococcus aureus* ATCC 2592) were used as test microorganisms. All of these bacteria were collected from International Centre for Diarrheal Disease Research, Bangladesh.

Disc-diffusion assay

Methanol extract of leaves of *Zingiber rubens* were subjected to antibacterial test using the disc-diffusion method as described by²⁶. Bacterial stock cultures were prepared by inoculating Mueller Hinton Agar plates with commercial bacteriological loops containing the test organisms, and then incubating the plates at 37 °C for 24 h. Microbial suspensions were prepared in nutrient broth media for bacteria, and incubated at 37 °C for 24 h. Turbidity of each culture was then adjusted to a similar optical density to that of McFarland 0.5. The standardized cultures were inoculated onto agar plates by swabbing uniformly over the entire surface of the medium. Sterile paper discs (6 mm diameter, made from Whatman No. 1 filter) impregnated with 10 µL of plant extract (10 -500 mg/mL) and a negative control- distilled water was then placed carefully on the surface of the inoculated agar plate with slight pressure. Standard antimicrobial disc (Amikacin 30 µg/disc) was used as positive control. The plates were then incubated at 37 °C for 24 hours for bacterial strains. Zone of inhibition including the disc was measured in mm as an indication of the antibacterial activity. All the test concentrations were run in triplicate.

Statistical analysis

The data from antidiarrheal, thrombolytic assay and antibacterial assay were expressed as Mean ± Standard Error Mean and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett ‘t’ test using SPSS software of 20 version. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

Phytochemical screening

Phytochemical composition of leaves of *Zingiber rubens* Roxb. are shown in Table 1. The therapeutic effects of plant materials generally result from the combination of secondary metabolites. These secondary metabolites are not only essential in the cell structure, but often are involved in the protection of plants against biotic and abiotic stresses. Natural products, as pure compounds or standardized extracts, provide unlimited opportunities for the drug discovery because of the unmatched availability of chemical diversity inside the plants. Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, seasons, climates, extracting solvent in plant and particular growth phases²⁷. Leaves are one of the highest sources of accumulation and are highly beneficial. The phytochemical screening showed that the leaves of *Zingiber rubens* contain reducing sugars, anthraquinones, flavonoids, terpenoids, saponins, tannins, alkaloids and cardiac glycosides. Plant extracts containing tannin, flavonoids, alkaloids, saponins and steroids have been reported to possess anti-diarrheal activity^{28,29}. Steroids have a wide variety of effects that affect the development, activity and plasticity of the nervous system and modulate pain³⁰. Saponins are natural detergents that prevent cholesterol reabsorption and thus reduces blood cholesterol levels in humans³¹. They also have anti-inflammatory, expectorant, immune stimulating, and antineoplastic effects³¹. Flavonoids are effective as antioxidant and antiplatelet^{32,33}. Glycosides are known to lower the blood pressure according to many reports³⁴.

Effect on castor oil- induced diarrhea

We evaluated the effect of methanol extract of leaves of *Z. rubens* on castor oil induced diarrhea. All mice from the control group (treated with vehicle) produced diarrhea after castor oil administration. The decrease in the severity of the diarrhea was measured by the evacuation index (EI). Treatment with different doses of ME before the cathartic agent (castor oil) significantly decreased liquid evacuation similar to the results produced by our positive control-loperamide. Negative control showed the evacuation index of 24 ($p < 0.001$) which was greatly reduced in case of positive control (5.14; $p < 0.001$). The trend in reduction in evacuation indices was also observed at 100 mg/kg, 200 mg/kg and 400 mg/kg of plant sample producing EI of

16.14, 15.57 ($p < 0.001$) and 12.71 respectively (Table 4). When calculating percentage of inhibition (Section 2.7), it was observed that the values were increased as the dose had been increased i.e. pre-treatment with ME of *Z. rubens* leaves (100, 200 and 400 mg/kg) induced a significant delay in the onset of diarrhea (33, 35 and 47% respectively, relative to the vehicle) as shown in Table 2 and Figure 1. Thus, the higher the dose of the extract (400 mg/kg), the better effect was observed compared to any of the groups. The result was in concord with two other species of same family; aqueous extract of rhizome powder of *Z. officinale* possesses good antidiarrheal activity which support the traditional use of the plant in the treatment of diarrhea³⁵. Also, diethyl ether extract of *Z. chrysanthum* rhizome demonstrated potent anti-diarrheal drug³⁶.

Thrombolytic activity

The effect of methanol extract of leaves of *Z. rubens* on in-vitro clot lysis are tabulated in Table 3 and Figure 2. Our present study was an attempt to compare the clot lysis potentiality of methanol extract of leaves of *Z. rubens*. Comparison of positive control (streptokinase) with negative control (water) clearly demonstrated that clot dissolution did not occur when water was added to the clot. Encouraged by the result of the positive control, we compared five different concentrations of the test sample with the negative control and observed significant thrombolytic activity. From Table 3, it is evident that percentage of clot lysis was 55.93% ($p < 0.001$) when 100 μ l of streptokinase (1,50,000 I.U.) was used as a positive control, while in the case of water (negative control) the percentage of clot lysis was negligible (1.20%) and highest concentration (10 mg/ml) of extract showed nearly equal potentiality (47.21%) as streptokinase (Figure 2). Percentage of clot lysis were increased in a dose-dependently manner for this plant extract. Percentage of clot lyses were 24.88 ($p < 0.05$), 32.24 ($p < 0.01$), 35.31, 39.59 ($p < 0.05$) and 47.21 ($p < 0.05$) for 2, 4, 6, 8 and 10 (all in mg/ml) respectively of extract (Figure 2). The values were found statistically significant tested by ANOVA followed by Dunnett's test. It was reported that phytochemicals like saponin, alkaloids, and tannin are responsible for the thrombolytic activity³⁷⁻³⁹. As methanol extract of this plant possess saponin, alkaloids; thus, similar to other species, those compounds may play role in demonstrating the thrombolytic activity of this plant. The result also agrees with previous reports where aqueous extract of *Z. cassumunar* rhizomes exhibited moderate thrombolytic activity⁴⁰ *Z. officinalis* was found to have strong inhibitory effect on platelet aggregation⁴¹.

Antibacterial activity

Disc-diffusion method was used to evaluate the antibacterial activity of the methanol extract of leaves of *Z. rubens* against two Gram-positive and two Gram-negative bacteria. Results of antibacterial activity are shown in Table 4. None of the concentrations of methanol extract of *Z. rubens* showed antibacterial activity against the test organisms *S. aureus*, *Micrococcus luteus*, *E. coli* and *P. aeruginosa*. Similar observation was also reported by Ghasemzadeh *et al.*⁴², where the stem extracts of *Z. zerumbet* did not show any antibacterial activity against *P.*

aeruginosa and *E. coli*. Leaf extract of *Z. spectabile* had no antibacterial effect whereas the rhizome extract of *Z. spectabile* exhibited weak activity against *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*⁴³. Diethyl ether extracts of *Z. officinale* showed resistance against *P. aeruginosa*, *Listeria monocytogenes*, *Salmonella typhimurium* and *E. coli*⁴⁴. In contrast to present study, aqueous leaf and rhizome extracts of *Z. zerumbet* showed mild antibacterial activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*) and Gram-negative (*Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*) bacteria strains⁴².

CONCLUSION

To the best of our knowledge, this is the first report about phytochemical screening and evaluation of *in vivo* antidiarrheal, in vitro thrombolytic and antibacterial activity of methanol extract of leaves of *Z. rubens*. The result of this study shows the presence of some phytochemicals which may be responsible for pharmacological effect. These findings suggest that the plant may be a potential source for the development of new antidiarrheal compound. Also the obtained results confirmed the presence of thrombolytic element in the leaves of *Z. rubens*; but no antibacterial activity which does not support the traditional use of this plant in various diseases caused by pathogenic microorganisms. The plant is widespread in different regions of Bangladesh. Thus, upon extensive chemical characterization of the leaves of this plant, it would be a great source of antidiarrheal and clot lysing compounds which could offer low-cost and easily obtainable drugs for these disorders to our countrymen.

CONFLICT OF INTEREST

The authors declare they have no competing interests.

ACKNOWLEDGEMENT

The authors would like to express their heartfelt obligation, indebtedness, gratitude and profound appreciation are made to Department of Pharmacy and Department of Microbiology, Noakhali Science and Technology University, Sonapur-3814, Noakhali, Bangladesh for their general facilities continuous support, untiring inspiration, scholastic supervision, constructive criticism, affectionate feeling and optimistic counseling for the conduction of this study.

REFERENCES

1. Rahman HMA, Ahmed K, Rasool MF, Imran I. Pharmacological evaluation of smooth muscle relaxant and cardiac-modulation potential of *Phyllanthus nodiflora* in ex-vivo and in-vivo experiments. *Asian Pacific Journal of Tropical Medicine*. 2017;10(12):1146–1153.
2. Hasan I, Hussain MS, Millat MS, et al. Ascertainment of pharmacological activities of *Allamanda neriifolia* Hook and *Aegialitis rotundifolia* Roxb used in

- Bangladesh: An in vitro study. *Journal of Traditional and Complementary Medicine*. Jan 2018;8(1):107-112.
3. Christenhusz MJM, Byng JW. The number of known plants species in the world and its annual increase. *Phytotaxa*. 2016;261(3):201-217.
 4. Phuaklee P, Sakpakdeejaroen I, Itharat A. Cytotoxic and antioxidant activities of two species of ginger extracts. *Thai Journal of Pharmacology*. 2010;32(1):82-85.
 5. Sirirugsa P. Thai Zingiberaceae : Species Diversity And Their Uses. Paper presented at: International Conference on Biodiversity and Bioresources: Conversion and Utilization; November 23-27, 1999; Phuket, Thailand.
 6. Xu Z, Chang L. Zingiberaceae. In: Xu Z, Chang L, eds. *Identification and Control of Common Weeds: Volume 3*. Singapore: Springer Link; 2017:909-911.
 7. Taylor TN, Taylor EL, Krings M. *Paleobotany- The Biology and Evolution of Fossil Plants*. 2nd ed. United States of America: Academic Press, Elsevier; 2009.
 8. Ahmed ZU. *Encyclopedia of Flora and Fauna of Bangladesh*. Asiatic Society of Bangladesh. 2008;12:1-505.
 9. Tripathi S, Singh KK. Taxonomic revision of the genus *Zingiber* Boehm. in North-East India. *Journal of Economic and Taxonomic Botany* 2006;30:520-532.
 10. Hynniewta SR, Kumar Y. The lesser-known medicine Ka Dawai Niangsohpet of the Khasis in Meghalaya, Northeast India. *Indian Journal of Traditional Knowledge*. 2010;9(3):475-479.
 11. Kayang H. Conservation of medicinal plants diversity of Meghalaya in India. In: Tandon P, Abrol YP, Kumaria S, eds. *Biodiversity and its Significance*. New Delhi, India: I. K. International Publishing House Pvt Ltd; 2007:233-253.
 12. Jantan I, Rafi IA, Jalil J. Platelet-activating factor (PAF) receptor-binding antagonist activity of Malaysian medicinal plants. *Phytomedicine*. Jan 2005;12(1-2):88-92.
 13. Hassan AM, Abutalib AA, Almagboul AZ, Kabbash AS. Antimicrobial activity of the rhizome essential oil of *Zingiber officinale* Roscoe *Advancement in Medicinal Plant Research*. 2017;5(1):5-10.
 14. Onyeagba RA, Ugbogu OC, Okeke CU, Iroakasi O, the So. Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). *African Journal of Biotechnology* 2004;3(10):552-554.
 15. Park M, Bae J, Lee DS. Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. *Phytotherapy Research* 2008;22(11):1446-1449.
 16. Dai DN, Thang TD, Chau LTM, Ogunwande I, A. Chemical constituents of the root essential oils of *Zingiber rubens* Roxb. and *Zingiber zerumbet* (L.) Smith. *American Journal of Plant Sciences*. 2013;4:7-10.
 17. Kantayos V, Paisooksantivatana Y. Antioxidant activity and selected chemical components of 10 *Zingiber* spp. in Thailand. *Journal of Developments in Sustainable Agriculture*. 2012;7(1):89-96.
 18. Dragstedt LR. Ethical considerations in the use and care of laboratory animals. *Academic Medicine*. 2002;77(2):144.
 19. Evans WC. *Trease and Evan's Pharmacognosy*. 16th ed. Nottingham, UK: Saunders Ltd.; 2009.
 20. Ayoola GA, Coker HAB, A. AS, et al. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*. 2008;7(3):1019-1024.
 21. Awouters F, Niemegeers CJ, Lenaerts FM, Janssen PA. Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. *Journey of Pharmacy and Pharmacology*. Jan 1978;30(1):41-45.
 22. Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M, Saha BP. Screening of anti-diarrhoeal profile of some plant extracts of a specific region of West Bengal, India. *Journal of Ethnopharmacology*. Feb 1998;60(1):85-89.
 23. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. *Thrombosis Journal*. Sep 12 2006;4:14.
 24. Kawsar MH, Sikder MA, Rana MS, Nimmi I, Rashid MA. Studies of thrombolytic and cytotoxic properties of two asteraceous plants of Bangladesh. *Bangladesh Pharmaceutical Journal*. 2011;14(2):103-106.
 25. Naderi GA, Asgary S, Jafarian A, Askari N, Behagh A, Aghdam RH. Fibrinolytic effects of *Ginkgo biloba* extract. *Experimental and Clinical Cardiology*. Summer 2005;10(2):85-87.
 26. Sacchetti G, Maietti S, Muzzoli M, et al. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry*. 2005;91(4):621-632.
 27. Bhat RS, Daihan DA. Phytochemical constituents and antibacterial activity of some green leafy vegetables. *Asian Pac J Trop Biomed*. 2014;4(3):189-193.
 28. Umer S, Tekewe A, Kebede N. Antidiarrhoeal and antimicrobial activity of *Calpurnia aurea* leaf extract. *BMC complementary and alternative medicine*. Jan 28 2013;13:21.
 29. Rahman MK, Barua S, Islam MF, et al. Studies on the anti-diarrheal properties of leaf extract of *Desmodium puchellum*. *Asian Pacific journal of tropical biomedicine*. Aug 2013;3(8):639-643; discussion 642-633.
 30. Mensah-Nyagan AG, Meyer L, Schaeffer V, Kibaly C, Patte-Mensah C. Evidence for a key role of steroids in the modulation of pain. *Psychoneuroendocrinology*. Dec 2009;34 Suppl 1:S169-177.
 31. Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y. Saponins from edible legumes: chemistry, processing, and health benefits. *Journal of medicinal food*. Spring 2004;7(1):67-78.

32. Cowan MM. Plant products as antimicrobial agents. *Clinical microbiology reviews*. Oct 1999;12(4):564-582.
33. Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Archives of biochemistry and biophysics*. Oct 1 1995;322(2):339-346.
34. Usunomena U, Ngozi OP. Phytochemical analysis and proximate composition of *Vernonia amygdalina*. *International Journal of Scientific World*. 2016;4(1):11-14.
35. Daswani P, Brijesh S, Tatali B, Antia NH, Birdi TJ. Antidiarrhoeal activity of *Zingiber officinale* (Rosc.). *Current Science*. 2010;28(2):222-229.
36. Arora M, Chandra M, Tewari H, et al. Study on *Zingiber chrysanthum rosc.* rhizome essential oil for its anthelmintic, anti-diarrheal and antibacterial activities. *European Journal of Pharmaceutical and Medical Research*. 2015;2(2):273-282.
37. Dewan SMR, Das A. Investigation of in vitro thrombolytic potential and phytochemical nature of *Crinum latifolium* L. leaves growing in coastal region of Bangladesh. *International Journal of Biological and Pharmaceutical Research*. 2013;4:1-7.
38. Gilman EF. *Allamanda neriifolia*. Gainesville, Florida: Institute of Food and Agricultural Sciences, University of Florida;1999.
39. Neu HC. The crisis in antibiotic resistance. *Science*. Aug 21 1992;257(5073):1064-1073.
40. Khobjai W, Sukati S, Jarmkom K, Eakwaropas P, Techaoei S. Evaluation of Thrombolytic Activity of *Zingiber cassumunar* Roxb. and Thai Herbal Prasaplai Formula *International Journal of Pharmacological and Pharmaceutical Sciences*. 2017;11(5).
41. Dhanik J, Arya N, Nand V. A Review on *Zingiber officinale*. *Journal of Pharmacognosy and Phytochemistry*. 2017;6(3):174-184.
42. Ghasemzadeh A, Jaafar HZ, Ashkani S, et al. Variation in secondary metabolite production as well as antioxidant and antibacterial activities of *Zingiber zerumbet* (L.) at different stages of growth. *BMC complementary and alternative medicine*. Mar 22 2016;16:104.
43. Sivasothyu Y, Awangu K, Ibrahim H, et al. Chemical composition and antibacterial activities of essential oils from *Zingiber spectabile* Griff. *Journal of Essential Oil Research*. 2012;24(3):305-313.
44. Awan UA, Ali S, Shahnawaz AM, et al. Biological activities of *Allium sativum* and *Zingiber officinale* extracts on clinically important bacterial pathogens, their phytochemical and FT-IR spectroscopic analysis. *Pakistan Journal of Pharmaceutical Sciences*. May 2017;30(3):729-745.