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Investigation of different pharmacological activity of *Boeica filiformis*

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Abstract
Boeica filiformis plant extract used to assess its different biological activity. Extract was made by soaking the dried plant powder in methanol. After comparing with the standard we found that Methanol extract of the sample gave the activity against all the experimented microbes of ZI (zone of inhibition) against *E.coli* and *B.subtillis.* After performing the antioxidant, thrombolytic, antidiarrheal, hypoglycemic and cytotoxic activity assay of methanol extract of sample plant we saw that it has a good biological activity that can be used as a potential traditional medicine.

Keywords: *Boeica filiformis,* antioxidant, antimicrobial, antidiarrheal, hypoglycemic, thrombolytic activity

Introduction
From the ancient time when chemical medicines were not introduced to people, traditional plants were reliable as they have healing properties to treat different diseases [5]. Over the past 50 years, many important drugs that have been revolutionized modern medicinal practice were isolated from traditional plants [3]. A study by WHO found that approximately 90% of the Ethiopian people use medicinal plants as their first choice [8]. Moreover, in developing countries almost 65-80% of population depends on medicinal plants primarily due to lack of accessibility of modern medicine and poverty [7]. Different part of the plants like leaf, root, bark is used for treatment because it is found that nature is one of source of 87% of drugs that can be used for treating all types of human diseases. About 25% of recommended drugs are obtained from traditional plant [2]. Different types of biological and pharmacological properties can be obtained from plants [6]. Now a day, medicinal plants are known as backbone of traditional medicine and more than 3.3 billion of the people use medicinal plants on regular basis [5]. In the developed countries, they are used to utilize medicinal plants over synthetic drugs [4]. For this reason, the researchers are screening different biological and pharmacological agents from natural source that can play an important role in the treatment of human diseases [3].
Boeica filiformis C.B. Clarke is a plant belongs to Gesneriaceae family. Gesneriaceae are a large family comprises 150 genera and 3000 species. Some species have been used in traditional medicine, mainly against fever, cough, colds, snakebite, pains, and infectious and inflammatory diseases [6]. It is an undershrub with small pink to purple flowers which are borne in loose-thread like panicles and are arising from the leaf axils [1]. Different parts of this plant like steam, leaf, bark have pharmacological properties that can deal with different diseases conditions [6]. Here, this study focus on the screening of different properties like antidiarrheal, hypoglycemic, antioxidant, thrombolytic, antimicrobial, cytotoxic that can be exerted by *Boeica filiformis*.

Methods and Materials
Collection of plant materials
The leaf part of Actinodephne angustifolia plant was collected in May, 2017 from Chittagong hill tract. After collection, the National Herbarium Bangladesh (NHB), Mirpur, and Dhaka authenticated the plant material and provided a plant identification number, which was 42929.

Preparation of the extract
At first, the leaves part was washed with fresh water to remove the unwanted dust particles and plant scrap. After that, the cleaned leaves were dried under the sun for a day. Then the leaves were again dried for 1 hour at 30-40◦C in hot air oven. By using a high capacity grinding machine, the dry and crusty leaves were ground. After that, at a normal ambient temperature (22-25◦C) around 900 g of ground powder was soaked in 2.5 L of methanol for a period of 2 days with occasional stirring. With the help of cotton filter (pore size: 110mm) filtration was done and rotary evaporator was used at 100 rpm at 30◦C to evaporate the maximum amount of solvent. For vaporizing the solvent completely from the extract, the leaf extract was kept under laminar airflow cabinet. Moreover, it was used to avoid any possibility of microbial growth in the extract while drying. Finally, 22.4 g of plant leaf extract was obtained and kept in dry and cool place and proper labeling was done. After that, this extract was used to conduct antioxidant, brine shrimp lethality assay, thrombolytic, antidiabetic, antimicrobial and hypoglycemic studies.

Chemicals
The chemicals were gallic acid [Sigma-Aldrich, USA], sodium chloride [Sigma-Aldrich, USA], Folin-Ciocalteu reagent [Sigma-Aldrich, USA], vincristine sulphate [Sigma-Aldrich, USA], 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) [Sigma-Aldrich, USA]., sodium carbonate [Merck, India] and ascorbic acid (ASA) [Merck, India], dimethyl sulfoxide (DMSO) [Fisher Scientific, UK]. Castor oil (WELL's Heath Care, Spain), 0.9% sodium chloride solution (normal saline)

(Orion Infusions Ltd., Bangladesh), charcoal meal (10% activated charcoal in 5% gum acacia), and loperamide (Square Pharmaceuticals Ltd., Bangladesh) were used for antidiarrheal activity test, and sodium chloride (Sigma) were used for cytotoxic activity test. All the chemicals used in this study were of analytical grade.

Anti-oxidant activity

Total phenolic content (TPC)

The phenols were oxidized by Folin-Ciocalteu in ionic phenolic solution. When the solution became yellow to dark blue, it is understood that the oxidation has been completed. after that, this color changed mixture measured in 760 nm in UV spectrophotometer. Finally, the value of the absorbance plotted in gallic acid calibration curve and data was evaluated as gallic acid equivalents (GAE).

Total Flavonoid content

Aluminum chloride was used to determine the total amount of flavonoids. Firstly, 0.5 ml of plant extract has been given a final volume of 1 ml ($MeOH/H_2O/CH_3COOH$=14:5:1) which was then mixed with Aluminum chloride reagent (4 ml, 133 mg of $AlCl_3 \times 6\ H_2O$ and 400 mg of CH_3COONa dissolved in 100 ml H_2O). After 5 minute, the absorbance was measured at 430 nm. Based on the calibration curve, total flavonoid content was calculated and it was expressed as gram equivalents.

DPPH free radical scavenging assay

The antioxidant activity of *Boeica filiformis* was determined by performing DPPH free radical scavenging assay. To run this assay, different concentrations of plant extracts were mixed with 2,2-diphenyl-1-picrylhydrazyl (DPHH) solution. In methanol or aqueous solution, free radicals were generated due to delocalization of the free electrons and a deep purple colored solution is produced. Then absorbance of different concentration solutions was measured at 517 nm in UV spectrophotometer. The decreasing value of DPHH at 517 nm is directly proportional to the radical scavenging activity.

Percentage of inhibition of DPHH free radical (1%) was calculated by using the following equation:

(1%) = (Absorbance of blank - Absorbance of sample)/Absorbance of blank ×100

50% of inhibition pf the extract concentration was calculated from the graph and the percentage of inhibition was plotted against extract concentration.

Cytotoxic activity

Brine shrimp lethality assay

In this assay, *Artemia salina* shrimp was used. Its offspring was hatched in replicated seawater to cultivate nauplii. Here, calculated amount of dimethyl-sulfoxide (DMSO) was added with sample and desired concentration of sample was prepared by dilution. The counted nauplii were placed in vials that contained approximately 5 mL simulated seawater with visual inspection. With the help of micropipette, various concentrations of samples were added to tubes. Here, vincristine sulfate was used as standard. The sample containing tubes were then placed in a dry place for 24 hours at room temperature. At the last, after 24 hours, the survived nauplii were counted. Percentage (%) of mortality was calculated by using the following equation:

Percentage of mortality= (Number of nauplii taken - Number of nauplii alive)/ Number of nauplii taken ×100

50% of lethal concentration of extract concentration was calculated from the graph plotted percentage of mortality against concentration.

Thrombolytic activity

The normal blood flow to the cells and tissues can be hampered due to thrombus as it blocks the blood vessel which can lead to lack of blood and oxygen. There are some thrombolytic medications like utokinase, clopifogrel, and streptokinase remove this thrombus and cells and tissues are remained in normal conditions. For this assay, fresh human blood was collected. Then, they were taken in three different pre-weighed sterile microbes and incubated for 45 minutes at 37°C. The upper fluid was entirely dispensed from all micro-tube lines when the clot was appeared. As a standard streptokinase was used and as a negative control distilled, water was used. 100 microliter of plant extract was taken in each tube and incubated for 90 minutes at 37°C. Next, liquid that was released from the clot was removed and the tubes were weighted again to observe the weight difference when the clot disruption occurred.

Percentage of clot lysis was calculated by following equation:

(%) of clot lysis = (released clot weighted) / (clot weight after clot disruption) ×100

Antimicrobial assay

Disc Diffusion Assay Method

In recent years, different studies are developing as antimicrobial agents to fight antibiotics resistance from different sources and highest concentration has given to screen and evaluate the antimicrobial activity. By using disc diffusion assay method, antimicrobial activity of *Boeica filiformis* was evaluated. *E. coli* bacteria (gram negative) and *Bacillus Subtilis* bacteria (gram positive) were used in this study. Mular Hinton agar (MHA) was used as media in this assay. Firstly, every petri dish was autoclaved for sterilization and 20 ml of MHA was poured in every petri dish. After that, the plates were kept for a time being to be settled. With the help of cotton swab, the nutrient broth of bacterial strains was incubated in MHA. Small disc of filter paper was made by using paper punch machine and then different concentrations of plant extract (200 mg/mL and 400 mg/mL) were used to swallow that filter paper. When the discs become dry, they were transferred to the petri dishes and kept in incubator for 24 hours at 37^0C. After 24 hours the zone of inhibition were calculated and for keeping the contamination limited, whole experiment was done under laminar flow.

Hypoglycemia activity

The hypoglycemic activity of the plant leaves was evaluated with glucose tolerance test. The test was done in two different ways which are orally and intraperitoneally.

Oral glucose tolerance test

In Oral glucose tolerance test, 24 healthy mice were fasted for 18 hrs. Then they were divided into four groups that contained six mice in each group. Here, 0.9% (w/v) normal saline was given to group I. Group II was received Glibenclamide (250 mg/kg). In addition, group IV and V was received methanol plant extract of 200 mg/kg and 400 mg/kg respectively. After 30 minutes, glucose (3g/kg) was

fed. After that at 0, 30, 90, and 120 minutes of glucose administration blood sample were taken from retro-orbital sinus and glucose level was estimated by glucose oxidase-peroxidase method.

Intraperitoneal glucose tolerance test

Initially 24 mice were fasted for 18 hours and then they were divided into four groups that contain six rats each. The group of negative control received only 0.9percentage (w/v) normal saline and standard group received Glibenclamide (250 mg/kg) while the samples were administered the plant extract (200 mg/kg and 400 mg/kg respectively). After 30 minutes, glucose solution (3g/kg) was injected intraperitoneally. At different time after giving glucose solution like t=0, t=30 minutes, t=90 minutes and t=120 minutes, blood sampling was taken and glucose level was determined by using glucose oxidase peroxidase method.

Antidiarrheal activity

Two different tests were conducted to evaluate the antidiarrheal activity of the experimented plant.

Castor Oil-Induced Diarrhea in Rats

Normal healthy 24 rats were fasted for 18 hours. The rates were divided into 4 groups (n=6). Group I was given normal saline (0.9% w/v) orally and Group II received Loperamide (5 mg/kg) as standard group. Groups III-IV received plant extract (200 and 400 mg/kg b. wt. respectively). After 1 hour, all groups received castor oil 1 mL each orally. Next, all the rats were placed in cages with adsorbent papers and observed for 4 hours for the presence of characteristic diarrheal droppings. 100% was considered as the total number of feces of control group. The activity was expressed as % of inhibition of diarrhea. The % of inhibition was measured by using following formula:

$$\text{Percent (\%) inhibition of defecation} = [(A-B)/A] \times 100$$

Where A is mean number of defecation time caused by castor oil and B is mean number of defecation time caused by drug or extract.

Magnesium sulfate induced diarrhea in rats

In the similar protocol as for castor oil induced diarrhea was followed for magnesium sulfate induced diarrhea. Initially, 24 healthy rats were fasted for 18 hours. The rats were divided into four groups that contained 6 rats each group. Normal saline (0.9% w/v) was given to group I. Loperamide (5 mg/kg) was given to group II and methanol plant extract (200 mg/kg and 400 mg/kg) was given to group III and IV respectively. After 60 minutes, 1 mL of magnesium sulfate solution was administrated orally and placed in cages lined with adsorbent papers and observed for 4 hours to see the presence of characteristic diarrheal dropping. 100% was considered as total number of feces of control group and % of inhibition was calculated.

Result and Discussion

Antioxidant activity

Total phenolic content (TPC)

In total phenolic content test, Gallic acid was used ad standard and methanol extract of leaves which was used as a sample. The absorbance of the sample plotted in Gallic acid calibration curve. The absorbance of the plant extract was found 0.719 and TPC value was 88.85 GAE/g against that absorbance which indicates that the plant has antioxidant activity.

Total Flavonoid content

The content of total flavonoid of the plant extracts was measured spectrophotometrically by using the aluminium chloride colorimetric assay. The flavonoid content of the extracts was expressed as mg quercetin equivalent per gram of the extract and it is 113.21 QE/g against the absorbance of 0.314 that indicates the present of flavonoid content.

DPPH free radical scavenging assay

It is known that DPPH free radical scavenging activity is increasing along with increasing concentration of the methanol extract. As the reference standard, ascorbic acid was used in this experiment for which IC_{50} value was 75.688 µg/ml. on the other hand, the IC_{50} value of the methanol extract of the sample plant was 707.29 µg/ml. this result indicates the presence of antioxidant activity which is less significant.

Table 1: Evaluation of DPPH free radical scavenging activity of methanol extract of *Boeica filiformis.*

	R^2 value	IC_{50}
Standard	0.6277	75.688
Sample (methanol extract)	0.6258	707.29

Cytotoxic activity

This brine shrimp lethality assay was used to assess the cytotoxic property of methanol extract of plant material. Here, different concentrations standard and sample were plotted that provided different percentages of mortality. Percentage of mortality was found to increase along with the increasing concentrations of standard and methanol extract. This study indicates the methanol extract of plant material has cytotoxic activity.

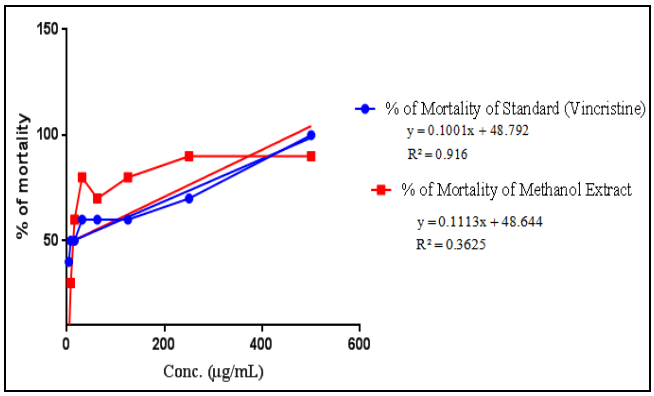

Fig 1

Thrombolytic activity

Table 2: Evaluation and results of the thrombolytic activity.

Name of the sample	W1	W2	W3	W4	W5	% of clot lysis
Plant extract	0.831	1.541	1.313	0.482	0.228	47.30
Standard	0.850	1.432	1.193	0.343	0.239	69.68
Blank	0.834	1.932	1.541	0.707	0.391	15.31

Here, W1 = Micro-tube weight, W2 = Clot with micro-tube weight, W3 = Clot with micro-tube weight after clot disruption, W4 = Clot weight after clot disruption, W5 = Released clot weight.

Plasminogen enzyme is usually activated by thrombolytic agents and it also removes fibrin bonds in blood, as a result, the clot becomes soluble and blood flow is restored. Here, methanol extract showed much lower level of thrombolytic activity than standard. Standard gave 69.68% clot lysis, distilled water was used as a negative control, which provided 15.31% clot lysis and methanol extract of plant leaves showed 47.30% clot lysis. After comparing the clots lysis value of plant extract with the positive control value, it was observed that plant material revealed thrombolytic activity but less than standard.

Antimicrobial assay

Table 3: Antimicrobial activity of the leaves of *Boeica filiformis.*

Group	Inhibition zone (mm)	
	Gram (-ve) bacteria *(E.coli)*	Gram (+ve) bacteria *(B. subtilis)*
Control	0.00	0.00
Standard	18 ± 1.414	22.333±0.577
Plant extract (200mg/mL)	0.00	0.00
Plant extract (400mg/mL)	14.667±1.528	17.667±0.577

The plant extract showed antimicrobial activity at all concentrations tested with a broad spectrum of activity, inhibiting against the growth of both Gram positive and Gram-negative bacteria. The antimicrobial potential was especially showed against *E. coli* and *B. subtilis*, when exposed to 400 mg/mL of methanol extract of plant and made it impossible when exposed to 200 mg/mL of methanol extract of dried leaves as shown in the table. These results indicate that the antimicrobial activity of the plant extract is not as significant as standard.

Hypoglycemia activity

Table 4: Oral glucose tolerance test in rats as a part of hypoglycemic activity of leaves of *Boeica filiformis.*

Group	Dose (mg/kg)	OGTT			
		Initial (mmol/L)	30 min. (mmol/L)	90 min. (mmol/L)	120 min. (mmol/L)
Control	_	5.017±0.703	17.967±1.970	12.067±2.199	6.484± 1.340
Standard	_	4.9±0.895	13.75±1.793	6.934±1.032796	2.5±0.352
MBF 200	200	4.35±0.644	19.4±4.751	11.717±3.725	3.917±0.896
MBF 400	400	4.67±0.686	20.034±6.386	9.277±2.836	2.884± 1.076

Table 5: Intra peritoneal glucose tolerance test in rats as a part of hypoglycemic activity of the leaves of *Boeica filiformis.*

Group	Dose (mg/kg)	IPGTT			
		Initial (mmol/L)	30 min. (mmol/L)	90 min. (mmol/L)	120 min. (mmol/L)
Control	_	21.367± 2.907	15.417±3.306	10.083±1.453	5.867±1.259
Standard		20.333±3.075	11.633±3.153	4.95±1.291	2.883±0.826
MBF 200	200	22.167± 1.699	17.117±2.662	9.483±2.104	4.567±1.0462
MBF 400	400	21.25±1.341	14.467±2.932	7.583±1.604	3.1±1.286

From the Table 4 and 5 we can say that our sample plant has the ability to act as a potential hypoglycemic medicine. Here MBF denotes methanol extract of *Boeica filiformis*. In both the cases which means in oral and intraperitoneal we saw that the administered glucose level go low as the time increases. If we compare them the intraperitoneal administration of glucose got a high blood glucose level at a short time and it went to low level at a short period of time.

Antidiarrheal activity

Table 6: Anti-diarrheal activity (castor oil induced diarrhea and $MgSO_4$ induced diarrhea) methanol extract of the leaves of *Boeica filiformis.*

Group	Dose (mg/kg)	Castrol oil induced diarrhea		$MgSO_4$ induced diarrhea	
		Total number of faeces in 4 hours	% of Inhibition	Total number of faeces in 4 hours	% of Inhibition
Control	_	18.167±1.471		16.167±4.070	
Standard	20	6.667±2.422	63.3	6.167±1.940	61.85
MBF200	200	10.167±1.602	44.04	9.334±2.338	42.27
MBF400	400	6.833±1.602	62.39	6.167±1.722	61.86

A significant reduction in the number of defecation instances was observed with all the test doses of the extract compared with the control group and standard group. There was graded reduction in intestinal fluid volume in graded MBF extracts. MBF (400 mg/kg) showed the reduction in the intestinal fluid volume with significant difference as compared with control group and standard group and % inhibition was 62.39% and 61.86% for castor oil induced diarrhea and magnesium sulfate induced diarrhea

Conclusion

The methanol extract of the *Boeica filiformis* leaf was

investigated to evaluate the therapeutic properties. In this study, it was clearly observed that this plant has various therapeutic potentials. The findings of the present study provide convincing evidence that methanol extract of *Boeica filiformis* leaves possesses remarkable antidiarrheal activity, cytotoxic effect, antioxidant activity, thrombolytic activity, antimicrobial activity, hypoglycemic activity. However, further chemical and pharmacological studies are required to isolate the bioactive compounds and elucidate the precise mechanisms responsible for the observed pharmacological activities of this plant.

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