

## COMPARATIVE STUDIES ON ANTIDIABETIC, ANALGESIC, AND CYTOTOXIC EFFECT OF ETHANOLIC EXTRACTS OF *AMARANTHUS GANGETICUS* L. AND *ALTERNANTHERA SESSILIS* L.

MD. MOHAIMENUL\*, KATHY DUTTA, NOWRIN FERDIOUSI, DEBENDRA NATH ROY

Department of Pharmacy, Jashore University of Science and Technology, Jashore, Bangladesh. Email: mohaimenulpharjust@gmail.com

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### ABSTRACT

**Objective:** The present study was designed to evaluate and compare antidiabetic, analgesic, and cytotoxic properties of Lal shak (*Amaranthus gangeticus* L.) and Chanchi shak (*Alternanthera sessilis* L.). We carried out this work to explore the medicinal uses of very common and cheap leafy plant vegetables among the people of all classes.

**Methods:** The antidiabetic activity was evaluated and compared by studying the effect of ethanolic extract of *A. gangeticus* (EEAG) and ethanolic extract of *A. sessilis* (EEAS) against blood glucose level of alloxan-induced diabetic mice in every 6 h for 24 h. To evaluate and compare analgesic and cytotoxic activity, different tests such as acetic acid-induced writhing test, hot plate test, and brine shrimp lethality bioassay test had been performed.

**Results:** The mice were treated with both plants extract at a dose of 200 mg/kg body weight in case of antidiabetic activity test. Blood glucose level was examined and found that there was a significant reduction of blood glucose level with EEAG ( $p < 0.05$ ) and EEAS ( $p < 0.001$ ) in comparison with their respective diabetic control group. Although both plant extracts reduced the blood glucose level, the glucose reducing effect was higher in EEAS. Both the plants showed significant ( $p < 0.05$ ) peripheral analgesic activity in treated mice but no significant central analgesic activity. EEAG showed higher peripheral analgesic activity than EEAS. In brine shrimp lethality bioassay, both the plants showed higher  $LC_{50}$  value thus cytotoxicity occurs at very higher dose and safe to administer.

**Conclusion:** In this study, both the plants showed sufficient antidiabetic property and higher  $LC_{50}$  value, thus administration of leafy vegetable Lal shak and Chanchi shak may be useful for diabetic people. Chanchi shak may be more helpful for diabetic people than Lal shak.

**Keywords:** *Amaranthus gangeticus* L., *Alternanthera sessilis* L., Antidiabetic, Analgesic, Cytotoxic.

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### INTRODUCTION

Plants have played a significant role in maintaining human health and improving the quality of human life [1]. In traditional medicine, there are numerous medicinal plants that have the potentiality to treat many diseases and disorders. Leafy vegetables are considered essential for well-balanced diets since they supply vitamins, minerals, dietary fiber, and phytochemicals. Having unique combination and amount of these phytonutraceuticals, each vegetable distinguishes from each other within their own group. To improve gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer, vegetables plays strong rule [2]. A vast majority of the population, particularly those living in rural areas, depends largely on medicinal plants and vegetable diet for the treatment of disease.

*Amaranthus gangeticus* L. belongs to the family Amaranthaceae with approximately 60 species that are acknowledged [3]. *A. gangeticus* L. originates from tropical Asia. In South and Southeast Asia (SEA), it is one of the major leaf vegetables and the most important *Amaranthus* species. *A. gangeticus* L. has mild spinach like flavor, high nutritive value, ability to grow in hot weather, and lower cost that made it a very popular vegetable. This plant is well known for its purple betalain pigments, such as amaranthine and isoamaranthine [4]. Goan/Indian folklore describes that the plant is a good liver tonic and therefore recommended as a vegetable for diabetic and anemic patients.

*Alternanthera sessilis* (L.) R. Br. DC. (Family: Amaranthaceae) is an annual, many branched herb. It can be erect or prostrate, rooting at the nodes, or may occasionally be a floating or emergent aquatic. The stems are greenish, pink to purplish, ribbed, up to 1 m, mostly glabrous except for tufts of

white hairs in the branch and leaf axils. The species occurs in tropical and subtropical regions. It is found all over the most of Africa, south of the Sahara and Egypt, throughout the Middle East, east through the Indian subcontinent, most Indian Ocean island groups. The leaves are used in eye diseases, cuts, wounds, and antidote to snake bite; skin diseases [5]. It is also reported about the wound healing property of *A. sessilis* L. [6]. The plants aerial parts also have shown a hepatoprotective activity [7].

Diabetes mellitus is a chronic and endocrine disorder caused by inherited and/or acquired deficiency in the production of insulin by the pancreas or by the inability of the insulin production. Type I diabetes (insulin dependent) is a condition characterized by insulin insufficiency because of lack of functional beta cells. Type II diabetes mellitus is a heterogeneous disorder characterized by a progressive decline in insulin action followed by the pancreatic beta cell dysfunction [8]. Complications such as renal failure, coronary artery disorder, cerebrovascular disease, neurological complications, blindness, dyslipidemia, obesity, limb amputation and failure of various organs, and eventually premature death are related with chronic hyperglycemia [9].

Pain and inflammation are reactions against detrimental stimuli, infection, trauma, or injury in the living tissues [10]. In most cases, pain and inflammation are treated by nonsteroidal anti-inflammatory drugs (NSAIDs). According to various medical literatures, various adverse reactions are known to be associated with the NSAIDs, thereby limiting the widespread use of these agents. Development of newer anti-inflammatory constituents possessing fewer side effects still remains a challenge to the scientific community.

The brine shrimp lethality assay is a general bioassay that seems to be capable of identifying a wide spectrum of bioactivity present in crude

extracts. The commercial availability of economical brine shrimp eggs, the low cost, the safety, and ease of performing the assay, as well as no special technology requirement make this a very helpful tool for the phytochemistry laboratory [11]. The method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in the small scale.

In this study, an attempt has been made to evaluate and compare the antidiabetic, analgesic, and cytotoxic effects of ethanolic extract of *A. gangeticus* (EEAG) and ethanolic extract of *A. sessilis* (EEAS) with a view toward elucidation and comparison of the probable claimed effects using various experimental models.

## METHODS

### Drugs and chemicals

Diclofenac sodium and metformin were procured from Square Pharmaceuticals Ltd., Bangladesh. Alloxan and vincristine sulfate were supplied by the Department of Pharmacy, Jashore University of Science and Technology (JUST) laboratory. All solvents and reagents used were of analytical grade and solvents obtained from Merck, Germany.

### Plant material

The Lal shak (*A. gangeticus* L.) and Chanchi shak (*A. sessilis* L.) were selected for this investigation. These were collected and identified from various areas of Jashore (23.1634° N, 89.2182° E) and Satkhira (22.3155° N, 89.1115° E), Bangladesh, during the month of October 2017.

### Preparation of plant extracts

The whole plant of *A. gangeticus* L. and aerial part of *A. sessilis* L. were thoroughly washed with fresh water to remove all contaminants and dried under shade at room temperature for a period of 9 days. Coarse powder of the materials was produced by grinding the materials with the help of a grinder. The coarsely powdered materials were weighted and about 500 mg of both powdered materials were macerated with ethanol (1:3) at room temperature for a period of 10 days with occasional shaking and stirring. The whole mixture was filtered through cotton and then Whatman No.1 filter papers. The solvent of the filtrate thus obtained was evaporated by rotary evaporator (Witeg, Germany, Model No: 2600000) to get the final extract (viscous mass). Then, the extract was stored in refrigerator (4°C) for further use.

### Experimental animals

A 4–5-week-old aged female *Swiss albino* mice of body weight 20–30 g (procured from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh) were used to run the antidiabetic and analgesic experiments. Before initiating the experiments, the animals were kept under standard environmental conditions, maintained 55–65% relative humidity, and exposed to alternative 12:12 h light and dark cycle at an ambient temperature of 26±2°C. Proper arrangement of foods and water *ad libitum* was ensured. Before the experiment, mice were habituated for 7 days in our laboratory environment and ensured the constant environmental and adequate nutritional conditions throughout the period of the experiment. *Artemia salina* Leach (brine eggs) was used for brine shrimp cytotoxicity test. This study was approved by ethical research committee of JUST, Jashore-7408, Bangladesh. The ethical approval number is ERC/FBS/JUST/2018-19.

### Antidiabetic activity test

#### Experimental induction of diabetes

Group animals were allowed to fast for 12 h and rendered diabetic by injection intraperitoneally with a freshly prepared solution of alloxan monohydrate (180 mg/kg body) [12] in normal saline after baseline glucose estimation was done. After alloxan induction, hypoglycemia may develop. To overcome alloxan-induced hypoglycemia, the alloxan-treated mice were allowed to food overnight. After 48 h, blood glucose concentration was measured using Rapid View™ blood glucose test meter using blood sample from the tail vein of the mice. The mice

having blood glucose level above 11.1 mmol/l were considered as diabetic mice and selected for the study.

### Experimental Design

Seven groups of mice, five in each received the following treatment schedule.

Group I: Normal control (saline 10 ml/kg p.o.)

Group II: Alloxan-treated control (180 mg/kg.ip)

Group III: Alloxan (180 mg/kg.ip) + *A. gangeticus* L. plants extract (200 mg/kg, p.o)

Group IV: Alloxan (180 mg/kg.ip) + *A. sessilis* L. plants extract (200mg/kg, p.o),

Group V: Alloxan (180 mg/kg.ip) + Standard drug, metformin (150 mg/kg, p.o)

Group VI: Normal *A. gangeticus* L. plants extract (200 mg/kg, p.o)

Group VII: Normal *A. sessilis* L. plants extract (200 mg/kg, p.o).

### Final observation

Both the plant extracts (200 mg/kg) and standard drug metformin (150 mg/kg) were given orally according to experimental design and blood glucose concentration was measured using Rapid View™ blood glucose test meter using blood sample from the tail vein of the mice in every 6 h for 24 h.

### Analgesic activity

#### Acetic acid-induced writhing test in mice

The test was conducted by modified method of Koster *et al.* [13].

Six groups of mice, five in each received the following treatment schedule.

Group I: Control. (10 ml/kg distilled water. p.o.)

Group II: *A. gangeticus* L. plants extract (200 mg/kg. p.o.)

Group III: *A. gangeticus* L. plants extract (400 mg/kg, p.o)

Group IV: *A. sessilis* L. plants extract (200 mg/kg, p.o)

Group V: *A. sessilis* L. plants extract (400 mg/kg, p.o)

Group VI: Diclofenac Na (100 mg/kg, p.o).

After 45 min of respective treatment, each mouse was injected intraperitoneally with 0.7% (v/v) acetic acid at a dose of 10 ml/kg body weight. Acetic acid is induced to provoke writhing activity. The writhing activity comprised constriction of the abdominal muscles together with a stretching of the hind limbs. The number of writhing responses of each mouse was counted for a 5-min period, which began 15 min late of acetic acid administration.

To determine the percentage of inhibition of writhing, the following formula was used.

$$\% \text{ Inhibition} = \frac{\text{Mean no of writhes}(\text{control}) - \text{mean no of writhes}(\text{test})}{\text{Mean no of writhes}(\text{control})} \times 100$$

### Hot plate test

Hot plate test was performed according to the method of Turner [14]. The method was used to evaluate the central mechanism of analgesic activity [15]. At first, mice were screened for this test by inserting them on a hot plate individually that was kept at 55±1°C. The mice that showing initial reaction time (difference of time between the placement of mice on hot plate and their responses to occur) of 15 s or less were selected for this study. During screening, the paw of mice may be damaged. A cutoff point of 15 s was used to overcome the problem of paw damage. Tramadol (10 mg/kg) was used as the standard drug. The mice were fasted for 16 h with water *ad libitum*. Thirty min before the treatment of each group, the response latencies of mice were recorded by placing them on hot plate after the observations of some parameters such as removal, jumping, or licking of the paws. The response latencies were also counted after 30, 60, 120, and 180 min of the respective treatment of each group.

Table 1: Effects of *A. gangeticus* L. and *A. sessilis* L. extract on blood glucose level in alloxan-induced diabetic mice

Time in hour	Blood glucose level in mmol/L						
	Non-diabetic mice		Diabetic mice			Non-diabetic mice	
	Normal control	Diabetic control	Alloxan +Metformin	Alloxan+ EEAG	Alloxan+ EEAS	Non-diabetic+EEAG	Non-diabetic+EEAS
0 h	6.28±0.65	19.95±1.61	24.66±2.98	22.17±2.70	18.12±2.44	5.18±0.88	8.15±0.93
6 h	4.94±0.45	20.0±1.66	11.42±2.51*	15.62±3.66	11.47±1.74*	3.32±0.41***	7.62±4.02*
12 h	4.88±0.32	19.92±1.055	17.94±4.54	23.70±3.84	16.47±3.03	4.48±0.42**	7.15±2.36*
18 h	4.74±0.12	20.25±1.55	11.62±3.92*	11.80±2.15*	10.27±3.43*	4.46±0.43***	5.12±1.84**
24 h	3.76±0.48	18.95±1.44	8.30±1.67***	14.35±2.40	6.87±1.07***	3.37±0.58***	6.17±1.55***

The blood glucose level was expressed as mean (±S.E.M.) values. Level of significance \*\*\*= p<0.001, \*\* = p<0.01, \* = p<0.05 compared to diabetic control. (ANOVA followed by Dunnett's t-test). EEAG: Ethanolic extract of *A. gangeticus* L., EEAS: Ethanolic extract of *A. sessilis* L. *A. gangeticus*: *Amaranthus gangeticus*, *A. sessilis*: *Alternanthera sessilis*

Table 2: Effects of *A. gangeticus* and *A. sessilis* on acetic acid-induced mice

Group	Dose	MW±SEM	% inhibition
Control	10 ml/kg	24.20±2.00998	0
Standard	100 mg/kg	13.20±2.53772**	45.45
EEAG-200	200 mg/kg	17±1.30384	29.75
EEAG-400	400 mg/kg	13.80±1.11355*	42.98
EEAS-200	200 mg/kg	15.40±4.10609*	36.36
EEAS-400	400 mg/kg	18±2.73861	25.62

The no. of writhing was expressed as mean (± S.E.M.) values. Level of significance \*\*\*= p<0.001, \*\* = p<0.01, \* = p<0.05 compared to control. (ANOVA followed by Dunnett's t-test). [MW: Mean writhing, SEM: Standard error mean, EEAG: Ethanolic extract of *A. gangeticus* L., EEAS: Ethanolic extract of *A. sessilis* L.] *A. gangeticus*: *Amaranthus gangeticus*, *A. sessilis*: *Alternanthera sessilis*

### Experimental design

Six groups of mice, five in each received the following treatment schedule.

- Group I: Control (10 ml/kg distilled water. p.o.)
- Group II: *A. gangeticus* L. plants extract (200 mg/kg, p.o.)
- Group III: *A. gangeticus* L. plants extract (400 mg/kg, p.o.)
- Group IV: *A. sessilis* L. plants extract (200 mg/kg, p.o.)
- Group V: *A. sessilis* L. plants extract (400 mg/kg, p.o.)
- Group VI: Tramadol (10 mg/kg, p.o.)

### Cytotoxic activity

#### Brine shrimp lethality bioassay

The brine shrimp lethality bioassay was carried out on EEAG and EEAS using the standard procedure of Meyer *et al.* [16]. To prepare simulated sea water (3.8% NaCl in water), 38 g NaCl was dissolved in 1 L of distilled water. A conical-shaped vessel containing previously prepared 1 L artificial seawater at 25–30°C temperature with a constant oxygen supply was used to hatching brine shrimp (*A. salina*) eggs. *A. salina* eggs took about 24–48 h to be hatched. Using a pipette, the larvae (nauplii) were collected. Dimethyl sulfoxide (DMSO, 1%) was used to dissolve the sample extract and the extract was then transferred into test tubes following the method of serial dilution in such way that each test tube contained 10 ml of artificial sea water having four different sample concentrations 800, 400, 200, and 100 µg/ml. Ten nauplii were placed in each test tube. DMSO (1%) was added to four test tubes. Vincristine sulfate was used as the positive control following the method of serial dilution in such way that each test tube contained 10 ml of artificial sea water having four different vincristine sulfate concentrations at 10, 5, 1, and 0.5 µg/ml. Again, ten nauplii were dropped in each test tube of positive control group. A negative control group was also tested having 10 nauplii in 10 ml sea water containing DMSO (1%). Following the period of 24 h at room temperature, the number of viable nauplii was counted.

### Statistical analysis

The analysis was performed using SPSS statistical package for WINDOWS (version 23.0; SPSS Inc., Chicago). For antidiabetic and analgesic tests,

the results of the study are expressed as mean±S.E.M and statistical significance between control and treated groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's t-test and levels at p<0.05 were considered significant, p<0.01 considered highly significant, and p<0.001 considered very highly significant compared to control. For brine shrimp lethality bioassay, LC<sub>50</sub> values were determined, based on the percentage mortality using Microsoft Office Excel scatter analysis.

## RESULTS

### Antidiabetic activity test

The result obtained from antidiabetic activity test is described in Table 1. Both the plant extracts were given at a dose of 200 mg/kg body weight and standard drug metformin was given at a dose of 150 mg/kg body weight. The effect of EEAG and EEAS on the blood glucose level of experimental mice was determined at every 6 h for 24 h. During 6 h observation, EEAS and metformin showed significant (p<0.05) reduction of blood glucose level with a mean value of 11.47±1.74\* mmol/L and 11.42±2.51\* mmol/L, respectively. At 18 h, EEAG, EEAS, and metformin also showed significant (p<0.05) reduction of blood glucose level with a mean value of 11.80±2.15\* mmol/L, 10.27±3.43\* mmol/L, and 11.62±3.92\* mmol/L, respectively. At 24 h, EEAS and metformin showed very highly significant (p<0.001) reduction of blood glucose level with a mean value of 6.87±1.07\*\*\* mmol/L and 8.30±1.67\*\*\* mmol/L, respectively. Thus, EEAS has more blood glucose reduction ability than EEAG.

### Analgesic activity

#### Acetic acid-induced writhing test in mice

The results of acetic acid-induced writhing test are displayed in Table 2. EEAG at a dose of 400 mg/kg and EEAS at a dose of 200 mg/kg body weight showed significant (p<0.05) analgesic effect with a mean value of 13.80±1.11355\* and 15.40±4.10609\*, respectively, with percentage of inhibition 42.98 and 36.36, respectively. Diclofenac sodium (100 mg/kg) used as standard drug which showed highly significant (p<0.01) analgesic effect with mean value of 13.20±2.53772\*\* and 45.45% inhibition.

### Hot plate test

The result of hot plate test is summarized in Table 3. This method is used to evaluate the central mechanism of analgesic activity. In this study, both the plant extracts were given 200 and 400 mg/kg body weight and standard tramadol was given as 10 mg/kg body weight orally. Both the plant extracts showed no significant analgesic effect.

### Cytotoxic activity

#### Brine shrimp lethality bioassay

Brine shrimp lethality activity of EEAG and EEAS is shown in Table 4. The lethality concentration (LC<sub>50</sub>) of EEAG is 1213 µg/ml and EEAS is 1364 µg/ml making those classified as non-toxic according to Meyer's toxicity index for BSLA [16]. None of the extract managed to achieve

Table 3: Effects of *A. gangeticus* L. and *A. sessilis* L. in hot plate test

Group	Dose	Response latency period(s)				
		0 min	30 min	60 min	120 min	180 min
Control	10 ml/kg	6.8920±2.07599	6.1720±1.03892	7.9680±2.9937	5.0020±6.1553	4.8000±9.4814
Standard	10 mg/kg	4±0.71*	3.41±0.40*	3.6±0.73*	3.52±0.5*	1.8±0.49**
EEAG200	200 mg/kg	6.2720±1.20011	5.7540±1.14492	3.2460±0.33992	4.3800±0.81997	4.4060±0.77272
EEAG400	400 mg/kg	5.9780±1.41151	5.0220±4.5817	5.3260±0.93404	4.2880±9.6103	4.9440±0.57867
EEAS200	200 mg/kg	8.7660±1.50797	6.0600±1.16816	6.2400±1.29631	3.0080±0.44125	5.1340±0.80834
EEAS400	400 mg/kg	5.3680±0.46645	8.3760±1.12082	5.0600±4.1662	3.6940±0.75662	5.2125±0.53266

The response latency period was expressed as mean (± S.E.M.) values. Level of significance \*\*\*= p<0.001, \*\* = p<0.01, \* = p<0.05 compared to control. (ANOVA followed by Dunnett's t-test). [MW: Mean writhing, SEM: Standard error mean, EEAG: Ethanolic extract of *A. gangeticus* L., EEAS: Ethanolic extract of *A. sessilis* L.]. *A. gangeticus*: *Amaranthus gangeticus*, *A. sessilis*: *Alternanthera sessilis*

Table 4: LC<sub>50</sub> of the *A. gangeticus* L. and *A. sessilis* L. in brine shrimp lethality bioassay

Test sample	Concentration (µg/ml)	% mortality	LC <sub>50</sub> (µg/ml)	Toxicity class
EEAG	100	10	1213	Non-toxic
	200	30		
	400	30		
EEAS	800	40	1364	Non-toxic
	100	10		
	200	20		
	400	30		
VS	800	30	0.93	Toxic
	0.5	40		
	1	50		
	5	90		
	10	100		

EEAG: Ethanolic extract of *A. gangeticus* L.; EEAS: Ethanolic extract of *A. sessilis* L.; VS: Vincristine sulfate. *A. gangeticus*: *Amaranthus gangeticus*, *A. sessilis*: *Alternanthera sessilis*

100% lethality even at the highest concentration 1000 µg/ml. Standard drug vincristine sulfate had a LC<sub>50</sub> value of 0.93 µg/ml.

## DISCUSSION

Diabetes mellitus is a chronic, endocrine disorder characterized by inability of cell to utilize insulin or by the ineffectiveness of the insulin production by pancreas. The International Diabetes Federation SEA Region, which composes of the countries of India, Sri Lanka, Bangladesh, Bhutan, Mauritius, and Maldives, has a high prevalence of diabetes. It has been reported that there were more than 72 million adults with diabetes in 2013, a number that is expected to exceed 123 million by 2035. Nearly 95% of people of the total diabetic patients have type 2 diabetes (T2DM) [17]. Moreover, another 24.3 million people have impaired glucose tolerance [18]. Both rural and urban population have reported increases in the number of diabetic people [19]. In a cross-sectional survey of 402 people among the urban middle class in Bangladesh, it has been reported that 35% had T2DM and 45% had metabolic syndrome [20]. Diabetes can progress to other disorders such as cardiovascular, kidney, and eye disorders. In fact, a high prevalence of chronic kidney disease has been seen following a community survey of urban people of Bangladesh, which has been correlated with insulin resistance [21].

We evaluate and compare the antidiabetic effect of EEAG and EEAS. In this study, we found that standard drug metformin reduced the blood glucose level from 24.66 to 8.30 mmol/L (±SEM) within 0–24 h. On the other hand, EEAG decreased blood glucose level from 22.17 to 14.35 mmol/L (±SEM) in alloxan-induced diabetic mice within 0–24 h and EEAS decreased blood glucose level from 18.12 to 6.87 mmol/L (±SEM) in alloxan-induced diabetic mice within 0–24 h which indicate that EEAS has higher hypoglycemic effect than EEAG (Table 1).

Acetic acid-induced writhing is a standard test for pain sensitivity to non-opiate analgesics. In writhing response experiments, the analgesic mechanism has been characterized as the release of different endogenous noxious mediators such as bradykinin, serotonin, histamine, and substance P [22,23]. In this study, EEAG at a dose 400 mg/kg showed significant reduction of number of writhing with a mean value 13.80±1.11355\* and percentage of inhibition showed 42.98%. EEAS at a dose 200 mg/kg also showed significant reduction of number of writhing with a mean value 15.40±4.10609\* and percentage of inhibition showed 36.36%. EEAG showed higher peripheral analgesic activity at higher dose and EEAS showed higher peripheral analgesic activity at lower dose. EEAG showed higher percentage of inhibition of writhing than EEAS (Table 2). Hot plate test is used to evaluate the central analgesic activity [13]. EEAG and EEAS have not showed any significant reduction of latency period in hot plate test (Table 3). Thus, it might be concluded that EEAG and EEAS have no central analgesic activity.

The rapid and simple brine shrimp lethality assay is considered as an economical bioassay for testing plant extracts bioactivity which in most cases associates considerably well with cytotoxic and anti-tumor properties [24]. The LC<sub>50</sub> value over 1000 µg/ml is considered as toxic while the LC<sub>50</sub> value below 1000 µg/ml is considered as non-toxic [16]. The LC<sub>50</sub> value showed by both the plant extracts prove that both the extracts are non-toxic thus safe to administer (Table 4).

## CONCLUSION

It can be concluded that both EEAG and EEAS showed its effectiveness as an antidiabetic and peripheral analgesic agent and EEAS has higher antidiabetic activity and EEAG has higher peripheral analgesic activity with high LC<sub>50</sub> value in brine shrimp assay indicating that it is safe to administer for people. We hope that further detailed investigation is underway to determine the exact bioactive phytoconstituents that are responsible for those activities of those plants. Moreover, it could be a potential source for novel "lead" discovery for antidiabetic and analgesic drug development.

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## AUTHORS' CONTRIBUTIONS

Md. Mohaimenul carried out the collection of plant, extraction process. Md. Mohaimenul, Kathy Dutta, and Nowrin Ferdiousi conducted the research work, conception and design of the study, statistical analysis and interpretation of data, and wrote the manuscript. Debendra Nath Roy supervised Md. Mohaimenul to conduct all the process. All authors read and approved the final manuscript.

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## CONFLICTS OF INTEREST

No conflicts of interest.

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