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Studies of phytochemical analysis, *in vitro* and *in vivo* evaluation of the local *Pseudoelephantopus spicatus* plant

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The plant *Pseudoelephantopus spicatus* is used in Bangladesh for its folkloric medicinal properties, particularly in rural and Chittagong Hill tract areas. Methanolic extracts of this plant and its various Kupchan partitionates (MESP, HSP, DCMSP, EtAcSP, and AQSP) were then subject to in vitro and in vivo bioassay investigations to determine their consistency and potency. The preclinical trial outcomes hold significance for advancing valid drug development methodologies. Qualitative phytochemical screening identified tannins, alkaloids, flavonoids, and steroids. The EtAcSP fraction of P. spicatus exhibited the highest phenolic content (102.56 mg of GAE/gm of extract). MESP displayed the most potent free radical scavenging activity (IC_{50} = 76.11 µg/ml on DPPH). Antimicrobial screening of the kupchan fraction revealed notable inhibition of bacterial growth by the DCMSP fraction, producing 6 to 16 mm zones of inhibition. Additionally, EtAcSP showed significant thrombolytic activity (290.16%). In toxicity assessments, DCMSP, HSP, AQSP, MESP, and EASP fractions exhibited brine shrimp lethality at varying concentrations. P. spicatus demonstrated promising results in peripheral and central analgesic, antidiarrheal, antidepressant, and hypoglycemic activities at doses of 400 and 200 mg/kg in Swiss-Albino mice. Moreover, the hexane extract analysis highlighted the herb's fatty acid composition, notably high in bound fatty acids (cis-9-oleic acid - 34.14%) and free fatty acids (Palmitoleic acid - 12.25%). This indicates higher unsaturated fatty acid proportions in bound form (BFAs) than in free form (FFAs). These findings suggest the potential bioactivity of P. spicatus, prompting further research to explore its potential as a folk medicine.

Key words: *Pseudoelephantopus spicatus*, total phenolic content, free radical scavenging activity, anti - diarrheal activity, thrombolytic activity, fatty acid.

INTRODUCTION

Amidst myriad sources, plants are regarded as a rich supply of substances that can be employed in the production of pharmaceuticals, whether they are natural or synthetic (Salmerón-Manzano et al., 2020). Folklore,

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also known as ethnopharmaceutical medicine, is particularly successful in treating common illnesses like bronchial asthma, fevers, piles, coated tongue, menstruation disorders, low sperm count, dysentery, and weak penile erection among people living in remote regions (Shoeb, 2019). Tobacco, coffee, alcohol, and other drugs as well as a number of treatments, all come from plants (Ren et al., 2021). As plants contain potential lead compounds and have been supplying myriad therapeutic agents for so many years, much advancement in pharmaceuticals has been extracted from natural plant sources (Baker et al., 1902). There are many medicinal plants in the world, and Plantago spicatus is recognized for its superior therapeutic properties (Leckie, 1972). The proximate composition, total amino acids, minerals, and a few nutritional variables of this plant that had been domesticated in south-east Asia were all examined. It was found that the plant contained 36.3, 13.8, 50.8% and corresponding amounts of crude protein, crude fat, ash, and nitrogen-free extractives. Phytochemical analysis of all the extracts revealed the presence of steroid, ethyl hexadecanoate, ethyl-9, 12-octadecadienoate, ethyl-(Z)-9-octadecenoate, ethyl octadecanoate, lupeol, stigmasterol, and stigmasterol glucoside. Furthermore, a class of terpenoids known as sesquiterpenes, which have cytotoxic effects, was also found in the Pseudoelphantopus genus. Also identified were high concentrations of isoleucine, histidine, cysteine and methionine, and threonine-four necessary amino acids. Furthermore, the flowers were found to be mineral-rich containing tannins (6.8%), L-DOPA (3.3%), hydrogen cyanide (0.017%), and phytic acid (1.5%) are present (Viswanathan et al., 1999). The hydroxylated germacranolides molephantin and molephantinin, both of which have cytotoxic and anticancer activity, have been found to be present in Piper spicatus. An earlier chemical analysis revealed that *P. spicatus* contains flavonoids, triterpenoids, flavonoid esters, sesquiterpene lactones, lupeol, elephantopin, triterpenes, and the stigmasterol epifriedelinol among its constituents (Mohan et al., 2010). The significant anticancer properties of sesquiterpene lactones make them particularly interesting for researchers. The entire plant is used traditionally to prevent diarrhea, and a medicine derived from the roots is used to treat colic. It has also been reported to be one of the foremost often used cough treatments in Middle America. In Philippine, this plant is widely accepted as an ethnobotanical drug for high blood pressure (HBP) and fever (Quiming et al., 2019). In Taiwan, the whole plant is employed to make the medicinal formulation “Teng-KhiaU”. It has been demonstrated that the water extracts of these three plants have a protective effect against acute liver damage caused by CC14 (Tsai and Lin., 1999). However, a systematic evaluation of the plant including pharmacognostical research is still needed. Bangladesh is home to a wide variety of floral species and a number of medicinal plant resources (Rahman et al., 2019). Thus, the plant used in the present study was collected from the Asteraceae family, known to possess a variety of bioactive compounds with antibacterial, antifungal, antioxidant, anticancer, and cytotoxic activities. While the flowers, roots, stem barks, and leaves of this plant have long been used in traditional medicine, the phytochemical properties of this plant remain largely unexplored.

MATERIALS AND METHODS

Collection of the plant and identification of the species

The plant (locally known as Dog's Tongue) was collected from Savar, Bangladesh. A taxonomic identification and accession number (DACB87366) was provided by the Bangladesh National Herbarium (BNH). The collected plants were cleaned from mud and dust particles and dried at room temperature and in an oven below 40°C. The dried plants were then ground to powder by a grinder (Cyclotec 200 meshes) and stored in an airtight bottle for further investigations.

Solvents and reagents

Merck (Germany) provided all solvents and analytical or laboratory-grade reagents used in the investigation. Before being used, the commercial solvents (Hexane, Dichloromethane, Ethyl acetate, and Methanol) were distilled.

Extraction

The plant's powdered (655 g) was taken in a clean flask and extracted with suitable methanol solvent systems for 15 days in an airtight container, with frequent shaking. During this period, the majority of the plant's extractable components were extracted into the solvent. The extract was then filtered through a filter paper funnel, and the filtrates were evaporated to dryness separately using a rotary evaporator (Stuart, UK) under reduced pressure at 40°C (Kupchan et al., 1973).

Phytochemical screening

Chemical tests were carried out on the aqueous extract and the powdered specimens to identify the phytochemical constituents. Different reagents and specific tests were used for different classes of compounds such as tannin, phlobotannin, alkaloid, saponin, flavonoid, steroid, terpenoid, and cardiac glycoside (Heller and Forkmann.1994; Mohon et al., 2010). The results are presented in Table 1.

Evaluation of antioxidant activity

Total phenolic content

Folin-Ciocalteu’s reagent was used with trace modification which gave Singleton and Rossi. 2.5 ml Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml Na_2CO_3 (7.5 percent w/v) solution were added to 0.5 ml extract solution (conc. 2 mg/ml). The mixture was incubated for 20 min at room temperature. After 20 min, a UV spectrophotometer was used to detect the absorbance at 760 nm, and the sample's total phenol content was determined using a

Table 1. Result of qualitative analysis for phytochemical screening of leaves of *Pseudelephantopus spicatus.*

Tannin	Phlabo-tannin	Alkaloid	Saponin	Flavonoid	Steroid	Terpenoid	Cardiac Glycoside
✔	✔	✔	✘	✔	✔	✘	✘

standard curve created from Gallic acid solutions of various strengths. Per gram of extract, the sample's phenolic content was estimated as mg of GAE (gallic acid equivalent) (Velioglu et al.1988).

Free radical scavenging activity (DPPH)

The plant extracts free radical scavenging activities (antioxidant capacity) on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were calculated where 3.0 ml of DPPH methanol solution (20 g/ml) and 2.0 ml of extract methanol solution at various concentrations were mixed (Brand-Williams et al.,1995). The antioxidant ability was studied using a UV spectrophotometer by comparing the bleaching of a purple-colored methanol solution of the DPPH radical by the plant extract to that of tert-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) (Süntar, 2020). After 30 min of reaction time at room temperature and in the dark, the absorbance was measured using a UV spectrophotometer at 517 nm against methanol as a blank. The following formula was used to compute the percentage of free radical DPPH inhibition:

$$I\% = \left(1 - \frac{A_{sample}}{A_{blank}}\right) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material). Extracts concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

Brine shrimp lethality bioassay

The methanol extracts of plant P.spicatus and its different partitionates, namely the Methanol Soluble Fraction, n-C6H14 Soluble partition, CH2Cl2 Soluble partition, Ethyl Acetate Soluble partition, and Aqueous Soluble partition, were investigated for the lethality in brine shrimp using a brine shrimp lethality bioassay (Hasan et al. 2019). The results of this study are presented in Table 4. To establish the fatal concentration (LC50) of the test samples after 24 h, a plot of the proportion of shrimps that perished against the logarithm of the sample concentration (toxicant concentration) was constructed, and a regression analysis was performed to calculate the best-fit line from the curve data.

Thrombolytic activity

The clot lysis method, as described in paper (Prasad et al., 2007) was used to extract methanol from the plant *P.spicatus* and its various partitionates. Healthy human blood was collected which was divided into 8 pre-weighed Eppendorf tubes (0.5 ml/tube) and warmed at 37 °C for 45 min. After the clot had formed, the serum was withdrawn entirely without disturbing the clot, then 100µl of streptokinase (SK), 100µl of distilled water and 100µl of various partitionates were introduced individually to each Eppendorf tube containing pre-weighed clot. These tubes with samples were again warmed at 37^0C for 90min and weighed again to see if there was a difference in weight after the clot was ruptured. The % clot lysis was determined by following equation:

$$\% \text{ Clot lysis} = \frac{Amount\ of\ Clot\ lysis}{Amount\ of\ Clot\ before\ lysis} \times 100\%$$

Anti-diarrheal activity

Castor oil induced diarrhea method introduced by Shoba et al. (2001; Rahman et al. 2020) with minor modifications, the approach was used in this investigation. Three groups of four mice each were used for the test, positive control, and control groups. Orally administered to the vehicle group was 10 ml/kg of vehicle (1 percent Tween 80 in normal saline). The positive control group was given 50mg/kg of loperamide orally. The methanolic extract of the plant *P. spicatusat* a dose of 200 mg/kg body weight was administered to the test group. Each animal was kept in a separate cage, and the floor lining was changed every hour. Each mouse received an oral dosage of castor oil to cause diarrhea after the previous treatment. Over the duration of a 5-hour observation period, the animals output of diarrheal excrement was counted.

Hypoglycemic activity

Due to hypoglycemic activity lowering the glucose level of blood of experimental animals (swiss-albino mice) was measured by following the procedure of Control and standard (Glibenclamide) group were administered orally by means of a long needle with a ball shaped and also the methanolic extract group orally on swiss-albino mice as making different types of dose level which methodology was described on article (Islam et al., 2019).

$$\%lowering\ of\ glucose\ in\ blood = \left(\frac{G_{control} - G_{test}}{G_{control}}\right) \times 100$$

Where, $G_{control}$ presents as glucose value in control group and G_{test} presents as glucose value in test sample group. After 60 min from completing the above procedure, all groups were ingested with 10% glucose solution (2gm/kg body wt.). The Glucose level was measured before, during, and after the administration of medicines at 0, 30, 60, 90 and 120 min.

Central analgesic activity

Central analgesic activity was observed by positive control tail flicking-immersion method which was illustrated in Chakraborty et al. (2018). The test swiss-albinos were fed methanolic extract orally, whereas the positive control rats were given morphine subcutaneously. The tails of mice were submerged up to 1-2cm in warm water held at (55±0.1)^{0}C. The response time of mice was taken to flick their tails known as pain reaction time. The latent time of tail-flicking response was measured before, during, and after the administration of medicines at 0, 30, 60, 90 and 120 min. The % elongation time of tail-flicking was calculated by this equation written below:

$$\% \text{ of time elongation} = \frac{T_{test} - T_{control}}{T_{control}} \times 100\%$$

Table 2. Test samples for total phenolic content determination.

Plant	Sample code	(mg of GAE/ gm of extractives±SD)
	MESP	55.49±1.88
	HSP	58.26±2.15
P. spicatus	DCMSP	58.29±2.21
	EtAcSP	104.34±1.74
	AQSP	98.31±2.22

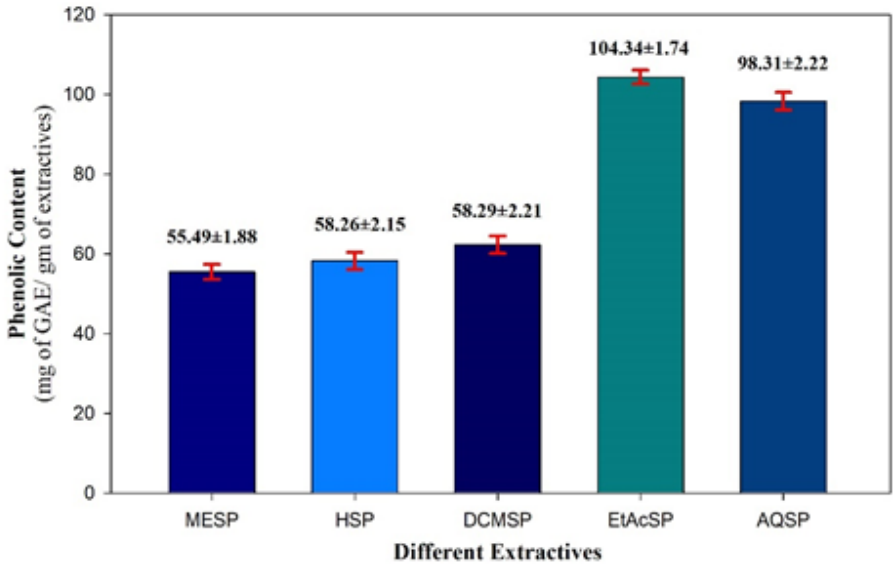

Figure 1. Plot of total phenolic content found in different extractes.

Fatty acid analysis

P. spicatus plant extract of n-hexane was taken for fatty acid analysis. The extracted fatty acids (Bound Fatty Acids (BFAs) and Free Fatty Acids (FFAs)) were converted into their respective methyl esters (Chowdhury et al., 2022).

RESULTS

Table 2 provides details of extractive values of phenolic content which demonstrate the phytochemical constituents of plants and also help in assuming actual constituents dissolve in a particular solvent. According to Figure 1 the amount of total phenolic content in MESP, HSP and DCMSP extractives is almost equal but lower than EtAcSP and AQSP extractives. Among all extractions of the plant *P. spicatus*in EtAcSP contains maximum phenolic content (104.34±1.74 mg of GAE/gm of extractives) followed by AQSP (98.31±2.22 mg of GAE/gm of extractives), DCMSP (58.29±2.21 mg of GAE/gm of extractives), HSP (58.26±2.15 mg of GAE/gm of extractives) and MESP (55.49±1.88 mg of GAE/gm of extractives). MESP = CH_3OH extract soluble partition, HSP = n-C_6H_{14} soluble fraction, DCMSP = CH_2Cl_2 soluble partition, EtAcSP = ethyl acetate soluble partition, AQSP = aqueous soluble partition of the *P. spicatus*. The antioxidant activity of IC_{50} values were obtained from Figure 2 in the DPPH method is different in different extractives and range from 28.31±2.10 µg/ml to 76.15±0.86 µg/ml. Among all extractives of *P. spicatus* the highest free radical scavenging activity was given by MESP (76.15±0.86 µg/ml) followed by HSP (75.92±0.87 µg/ml), DCMSP (66.13±1.06 µg/ml), AQSP (28.47±1.03 µg/ml) and EtAcSP (28.31±2.10 µg/ml) as compared to BHT was 6.96±0.54 µg/ml (Table 3). In bioassay of Brine shrimp lethality, LC_{50} values obtained from Figure 4 by measuring logarithmic concentration at 50% mortality. Table 4 reflected that the extractive of plant *P. spicatus*the highest brine shrimp lethality was found by Ethyl acetate Soluble Partition 411.71±2.79 µg/ml followed by CH_3OH Soluble Partition 329.53±8.34 µg/ml, Aqueous Soluble Partition 112.15±4.50 µg/ml, CH_2Cl_2 Soluble Partition 96.55±2.79 µg/ml and n-C_6H_{14} Soluble Partition 90.10±4.21 µg/ml (Figure 3). Figure 5 depicted that over all the extract has a higher LC_{50} value as compared to Vincristine sulfate.

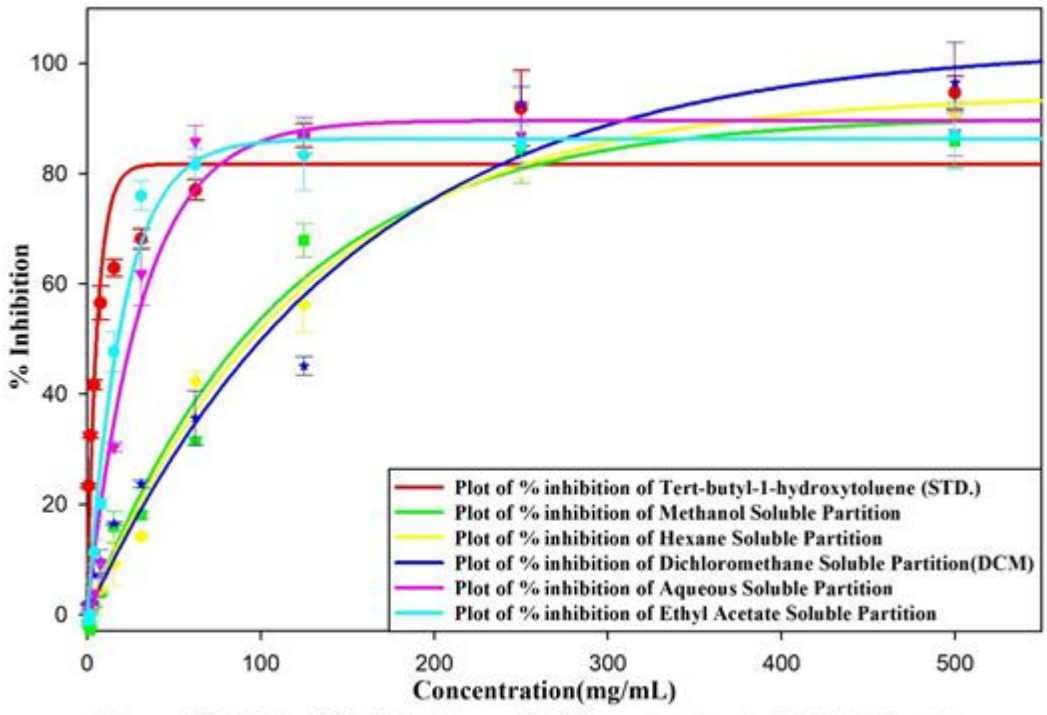

Figure 2. Plot of % inhibition of different solvents soluble partition.

Table 3. IC_{50} values of the standard and partitionates of plant P.spicatus.

Plant	Sample Code	(IC_{50}±SD µg/ml)
P. spicatus	BHT	6.96±0.54
	MESP	76.15±0.86
	HSP	75.92±0.87
	DCMSP	66.13±1.06
	EASP	28.31±2.10
	AQSP	28.47±1.03

Table 4. LC_{50} values of the test samples of plant P. spicatus.

Plant	Test samples LC_{50} ±SD (µg/ml)	
P.spicatus	VS	9.02±0.23
	MESP	329.53±8.34
	HSP	90.10±4.21
	DCMSP	96.55±2.79
	EtAcSP	411.71±2.79
	AQSP	112.15±4.50

The investigation of thrombolytic potential is merely observational research, and before drawing any conclusions, phytochemical and pharmaceutical potentials of the extract should be further explored. Table 5 provided information about the Ethyl Acetate Soluble Partition (EtAcSP) of the plant *P. spicatus* exhibited dramatically high thrombolytic activity 290.38±9.48% but rest of all soluble partition of extracts were showed the value of % of clot lysis in between Blank and SK (streptokinase) soluble partitions. In terms of % of clot

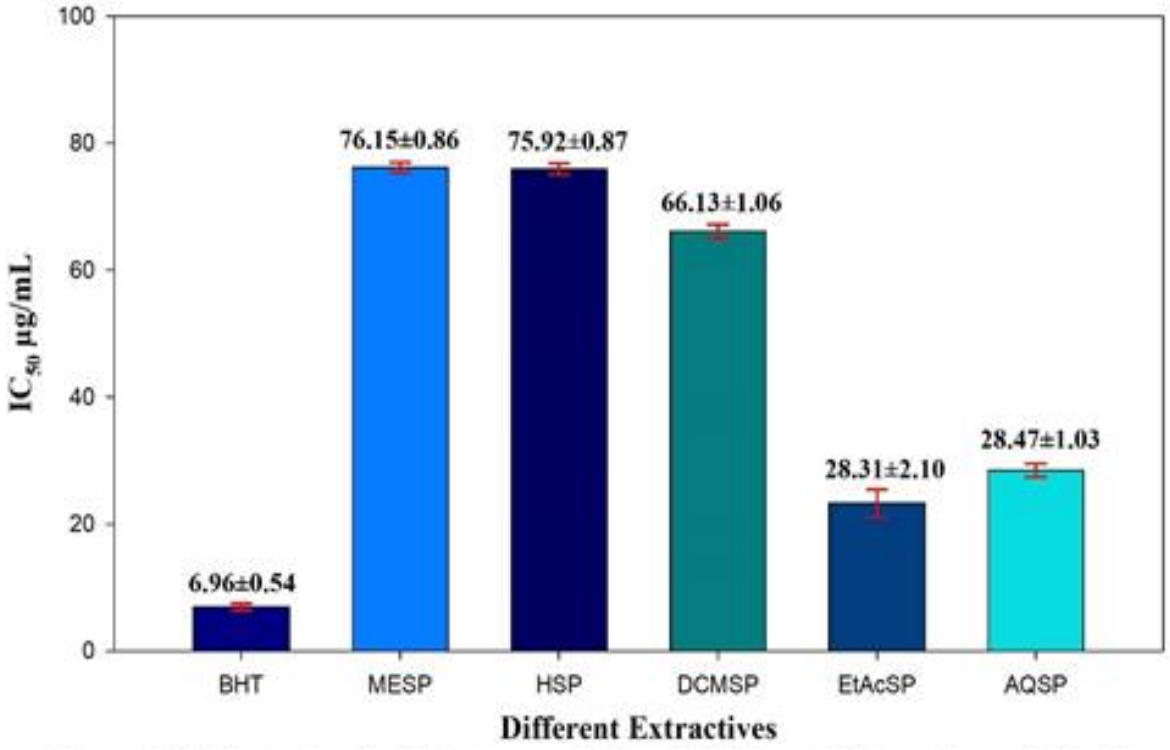

Figure 3. Plot of antioxidant capacities of different extractives of P.spitacus in terms of IC_{50} values with standard deviation

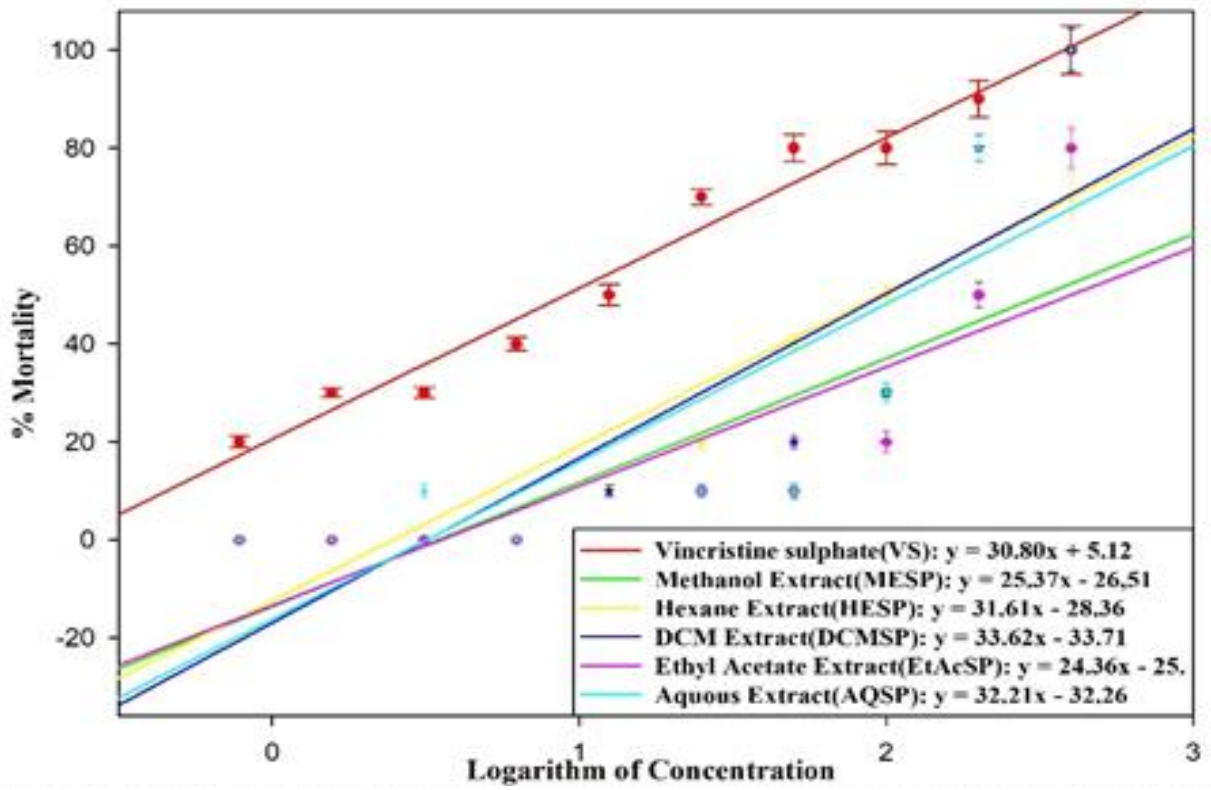

Figure 4. Plot of % mortality and predicted regression line of soluble partition of various solvent.

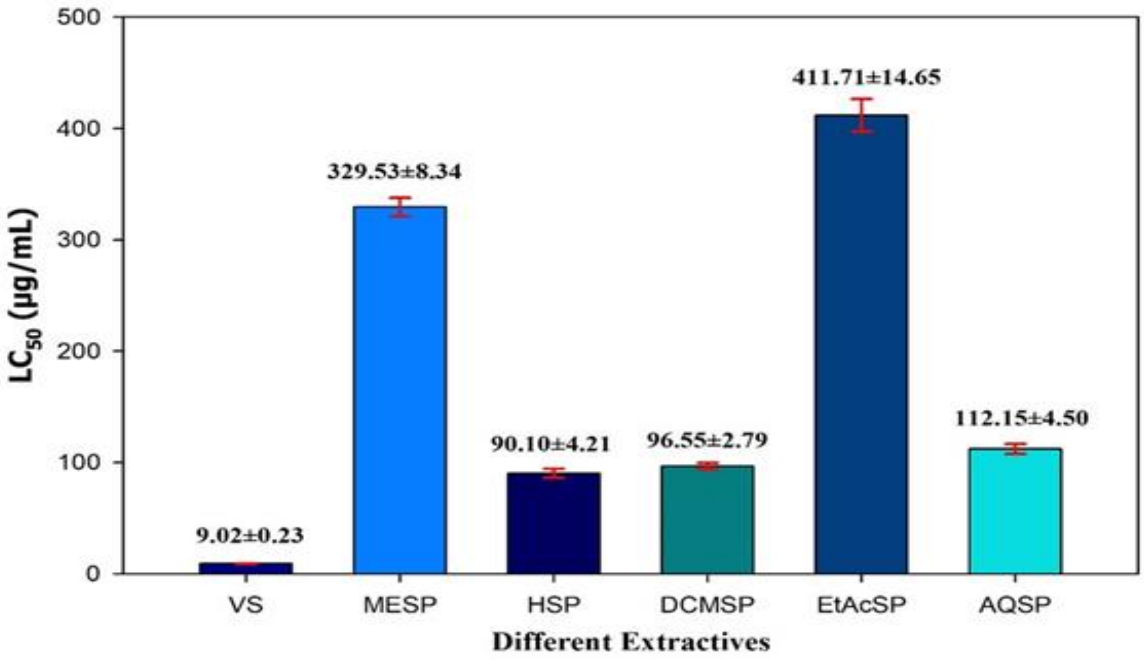

Figure 5. Plot for LC_{50} values of different extractives of *P. spitacus* plants.

Table 5. Thrombolytic Activity (in terms of % of clot lysis) of the extractives of the plant *P. spicatus.*

Plants	Fractions name	% of clot lysis±SD
P. spicatus	MESP	52.39±1.82
	HSP	33.51±2.00
	DCMSP	59.24±2.09
	EtAcSP	290.38±9.48
	AQSP	35.27±2.26
	Blank	8.58±1.24
	SK	65.25±2.25

lysis, the extractives of the plant was found CH_2Cl_2 Soluble Partition (DCMSP) 59.24±2.09%, CH_3OH Soluble Partition (MESP) 52.39±1.82%, Aqueous Soluble Partition (AQSP) 35.27±2.26% and n-C_6H_{14} Soluble Fraction (HSP) 33.51±2.00% thrombolytic activity. Similarly, Figure 6 was depicted as same as Table 5 described that the peak of EtAc soluble partition significantly high. The study's goal was to see how effective methanolic extracts of the plant *P. spicatus* were as anti-diarrheal medicines. The statistical analysis of this study was showed in Table 6 that the methanolic extract of plant *P. spicatus* soluble fractionate possesses statistically significant anti-diarrheal activity. Further study, however, is necessary to make final conclusion. The effects of methanolic extract of plant *P. spicatus* at 200 and 400 mg/kg dose to lower blood glucose level were observed as follows to evaluate their hypoglycemic activity (Table 7). The information reflected from Table 8 that the methanolic extract of plant *P. spicatus* has no statistically significant anti-diabetic action at dose of 400/200 mg/kg. Statistical evaluation of T-test and P-test value obtained from the data of different duration times after the processing experiment was confirmed that the extract of methanol of plant *P. spicatus* at doses of 200 and 400 mg/Kg responds differently with time. A lucid concept was depicted by Figure 7, among all over the time period of experiments only the case of the data obtained after 60, 90 and 120 min are extremely statistically significant except 30 min.

According to the data in the Table 9 it was reflected that % of elongation of the crude extracts of plant *P. spicatus* at dose of 400 and 200 mg/kg exhibited mild analgesic activity compared to standard doses of morphine at 30 min after administration of sample. But these doses showed remarkable analgesic activity when gradually the period of time was extended as Table 9

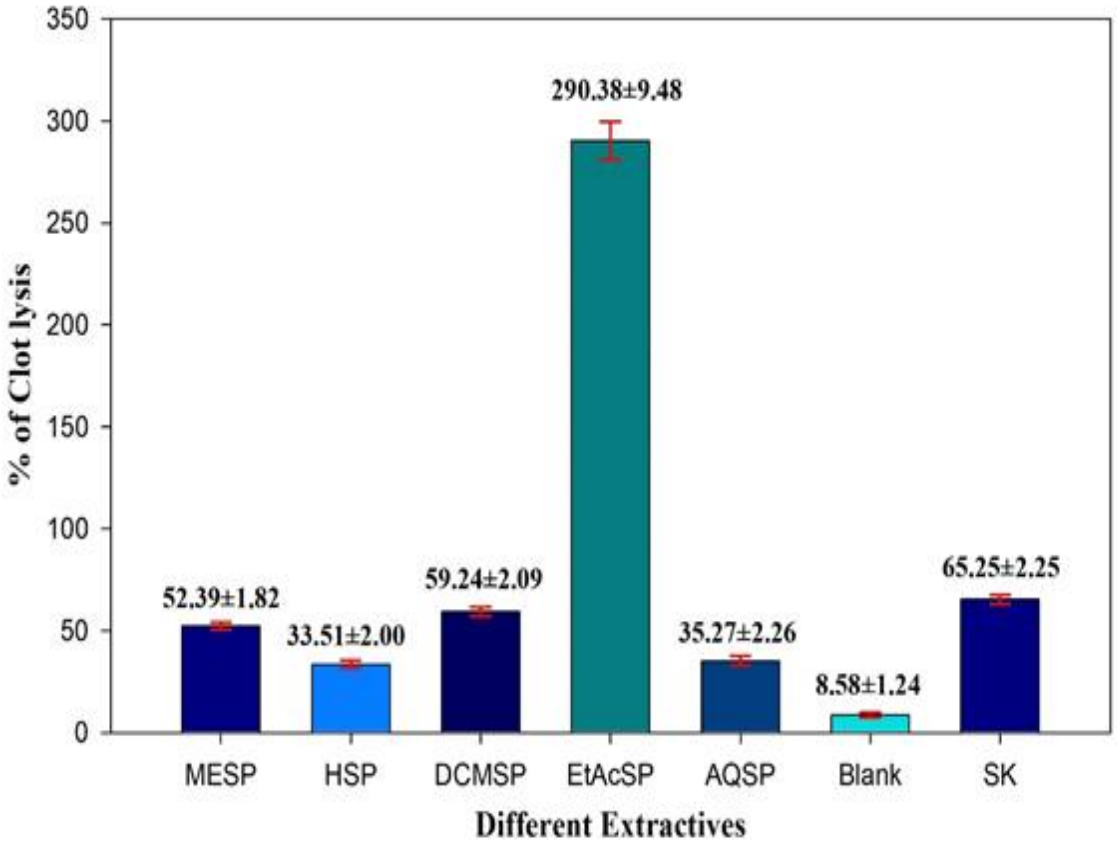

Figure 6. Plot of thrombolytic activity of different extractives of *P. spitacus* in terms of % clot lysis with standard deviation.

Table 6. Statistical data for castor oil (1mL/mice) induced diarrhea in mice due to the effect of different concentration of methanolic extract of plant *P. spicatus.*

Code No.	Number of diarrheal faeces (Mean)	% Reduction of diarrhea	t-Test value	Standard error of mean	P value	Level of significance
CTR	10.75			0.48		
STD	2.75	74.41	11.816	0.48	0.0001	Extremely statistically significant
MESP (400)	3.00	72.09	10.333	0.58	0.0001	Extremely Statistically significant
MESP (200)	4.75	55.81	4.482	1.25	0.0042	Very Statistically significant

Table 7. Data for activity hypoglycemic behavior of crude extract of plant *P. spicatus.*

Code no.	Plasma level of glucose (MeanH)				
	00 min	30 min	60 min	120 min	180 min
CTL	6.1	18.2	14.6	8.6	7.7
STD	6.3	13.4	12.0	7.9	4.8
MESP (400)	6.6	14.7	11.4	8.3	7.0
MESP (200)	7.2	13.6	10.5	7.3	5.4

Table 8. Statistical evaluation of the Data of activity hypoglycemic behavior.

Code No.	t Test value	Standard deviation (SD)	Standard error (SEM)	P value	Level of significance
CTR		5.134	2.296		
STD	0.7640	3.689	1.650	0.4668	Not statistically significant
MESP (400) Average	0.5221	3.417	1.528	0.6158	Not statistically significant
MESP (200) Average	0.8241	3.252	1.454	0.4337	Not statistically significant

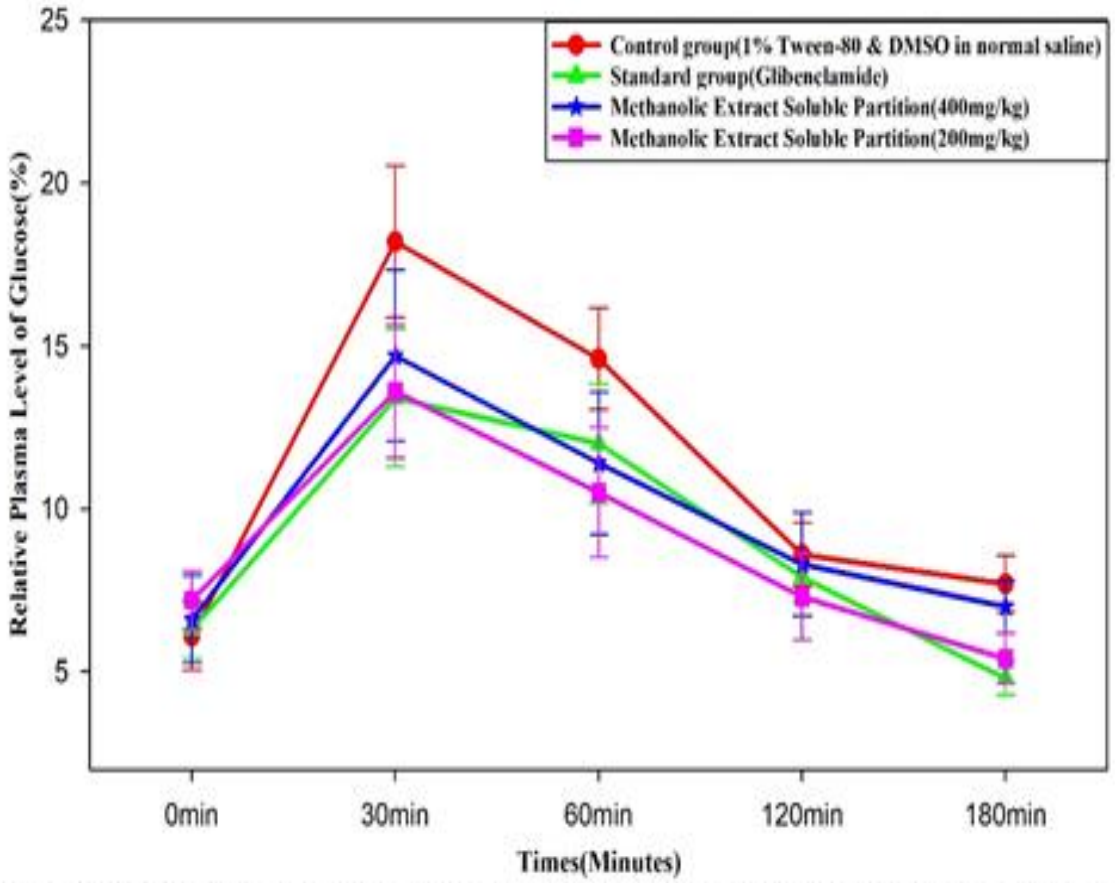

Figure 7. Plot of plasma level of glucose (mmol/L) of mice at different time for the evaluation of hypoglycemic activity.

Table 9. Central analgesic activity of the root extractives of *B. ceiba* in Swiss-Albino mice.

Animal Group	Average immersion time							
	30 min		60 min		90 min		120 min	
	Average time of immersion ± SD	% Elongation ±SD	Average time of immersion ± SD	% Elongation ±SD	Average time of immersion ± SD	% Elongation ±SD	Average time of immersion ± SD	% Elongation ±SD
CTRL	2.08 ± 0.22		2.56±0.23		3.39±0.49		2.71±0.37	
STD	2.09 ± 0.48	0.480±0.48	6.86±0.36	168.36±7.93	10.68±2.37	213.71±12.07	16.07±3.78	492.55±17.87
MESP (400)	2.10 ± 0.29	1.057±0.16	5.49±0.41	114.78±4.98	7.17±0.60	111.29±3.07	10.31±1.40	280.23±11.02
MESP (200)	2.78 ± 0.73	33.41±2.38	4.28±0.66	67.18±4.02	6.43±0.52	89.56±4.02	8.39±2.81	209.55±11.79

exhibited highest 280.23±11.02% of elongation at 120 min in 400 mg/kg dose. It was also observed that high doses of 400 mg/kg were given better response in central analgesic activity compared to low doses of 200 mg/kg (Figure 8). Moreover, the data of statistical analysis was obtained after different periods of time min for central analgesic activity of Table 10 provide the information about that accept 30 min time period all the study at 60, 90 and 120 min gives extremely statistically significant results. The amount of hexane extract was 502 mg. Free fatty acid was obtained 49.7 mg and Bound fatty acid was obtained 90.2 mg. the relative percentage and the amount of free fatty acid and bound fatty acid in g/100 g of dry powder was given in Table 11. The analysis of bound fatty acids (Table 10) showed that the hexane extract of plant *P. spicatus* contains highest proportion of Cis-9-Oleic acid (34.14%) followed by octadecanoic acid (28.36%) and lowest proportion of Palmotoleic acid (26.13%). The analysis of free fatty acids (Table 11) showed the highest proportion of Palmitoleic acid (12.25%) and lowest proportion of Octanoic acid (11.52%). This finding indicates that saturated fatty acids are higher in proportion as bound fatty acids than that of free fatty acid.

DISCUSSION

In this study, a survey was conducted to search for antioxidants present in the *P. spitacus* plant. The analysis of the partitionates of *P. spitacus* revealed the presence of decent amounts of phenolic compounds, which act as antioxidants and have a mitigating power. A DPPH and antioxidant activity study were conducted, which gave remarkable results. Furthermore, the Brine shrimp lethality test was conducted to determine the toxicity of the plant in order to assess its suitability in preventing the uncontrolled cell growth observed in higher living organisms, such as cancer. The test described the lethal ability of plant extract on a living organism as well as the probability of having anti-cancer activity (Fernández et al., 2020). In this study, all the extracts showed low toxicity as their LC50 values were found to be relatively high, except for the Vincristine Sulphate extract. Thus, this result suggests that the plant does not have any side effects of cytotoxicity. Additionally, a thrombolytic study was conducted with the plant extract to determine its potential for breaking down blood clots. The effectiveness of lead compound-containing plant extracts in liquefying blood clots of living organisms was studied through thrombolytic analysis (Maqsood et al., 2021). The experiment showed that *P. spicatus* had a good amount of % clot lysis of blood, indicating that the partitionates had a significant effect on thrombolytic activity. This suggests that the plant could be used for the therapy of heart and brain stroke patients, although further research into this would be necessary. Phytochemical screening of the methanolic extracts confirmed the presence of secondary metabolites such as tannins, alkaloids, flavonoids, and steroids, which play an important role in preventing malfunctions (Mekonnen et al., 2018). Tannins, in particular, are absorbed in the intestinal mucosa to form protein tannates, which exhibit anti-diarrheal activity (Bilić-Šobot et al., 2016). The present experiment investigated the potential of *P. spitacus* to reduce diarrheal activity in swiss-albino mice with different concentrations of doses. Results indicate that the dose of 400mg/kg was the most effective at reducing diarrheal activity. Secondary metabolites in the extracts may also have potential hypoglycemic effects. Diabetes is a growing problem worldwide, and *P. spitacus* was studied for its possible hypoglycemic function in the swiss-albino mice model. However, statistical results showed that

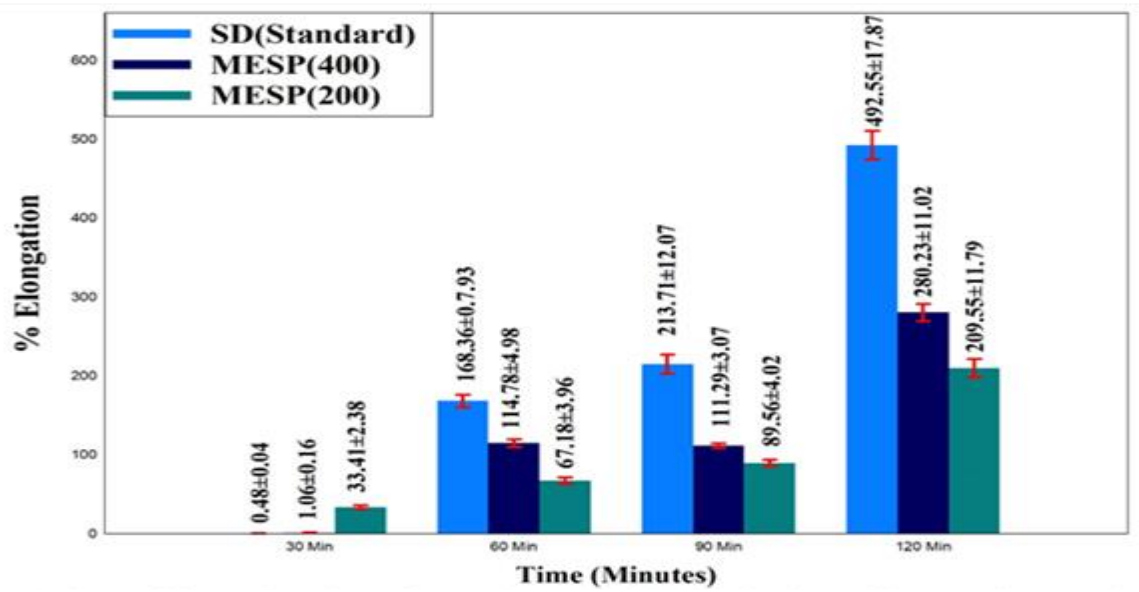

Figure 8. Evaluation of central analgesic activity by tail immersion method of methanolic extract of plant *P. spicatus.*

Table 10. Evaluation of the statistical data obtained after 30, 60, 90 and 120 min for central analgesic activity.

Period	Group			
	STD		**MESP (400)**	**MESP(200)**
Data Obtained after 30 Min	t-Test	0.0373	0.1221	1.8102
	p-Test	0.9715	0.9068	0.1202
	Level of significance	Not statistically significant	Not statistically significant	Not statistically significant
Data Obtained after 60 Min	t-Test	19.8197	12.3517	4.9072
	p-Test	0.0001	0.0001	0.0027
	Level of significance	Extremely statistically significant	Extremely statistically significant	statistically significant
Data Obtained after 90 Min	t-Test	5.9985	9.7121	8.4635
	p-Test	0.0010	0.0001	0.0001
	Level of significance	statistically significant	Extremely statistically significant	Extremely statistically significant
Data Obtained after 120 Min	t-Test	7.0298	10.4597	4.0063
	p-Test	0.0004	0.0001	0.0071
	Level of significance	Extremely statistically significant	Extremely statistically significant	statistically significant

Table 11. Amount and relative percentages of BFA and FFA.

Amount of Hexane extract*	Amount of Bound fatty acids (BFA)*	Amount of Free fatty acids (FFA)*	Name of fatty acids	Bound fatty acids (Relative %)	Free fatty acids (Relative %)
0.502	0.0902	0.0497	Octanoic		11.52
			Palmitoleic	26.13	12.25
			Octadecanoic	28.36	
			cis-9-oleic	34.14	

*Value expressed in g/100 g of dry powder.

the methanolic extracts did not have any significant hypoglycemic effects in this model. Central analgesic drugs are usually used to treat body tissue in living organisms (Bukhari, 2013). In this research, the central analgesic activity of crude extracts from *P. spitacus* plants was experimentally investigated to identify active compounds that can generate an analgesic effect at the location of the pain. Statistical analysis indicated that *P. spitacus* plants had a significant analgesic effect that grew slowly over time, revealing a highly significant activity after 60, 90 and 120 min. However, further research is needed to draw firm conclusions. Fatty acid analysis revealed that the amount of bound fatty acids (BFAs) was higher than free fatty acids (FFAs), suggesting that small amounts of fats and oils could be broken down into FFAs by hydrolysis (Chowdhury et al., 2022). The analysis also found that unsaturated fatty acids dominated over saturated fatty acids in both BFAs and FFAs, meaning that consuming *P. spicatus* will not produce any adverse effects. Octadecanoic acid (stearic acid) was found to be present at 28.36%. Therefore, this plant can also be utilized for cosmetic applications.

Conclusion

Experimental findings from the study concluded that *P. spicatush* as a significant amount of phenolic content which exhibited great antioxidant activity, free radical scavenging activity, and brine shrimp lethality, indicating that consumption of this plant is not harmful due to the absence of toxicity. The DCM extract has good antimicrobial activity, while the ethyl acetate extract exhibited good thrombolytic activity, which could be used for essential treatments of CVST patients. This research also concluded that *P. spicatush* as statistically significant anti-diarrheal activity, hypoglycemic activity, central and peripheral analgesic activity and mild depressant activity in mice. Palmitoleic, cis-9-oleic acid, octadecanoic acid, and octanoic acid were identified as major fatty acid constituents of this plant. This primary research suggests that *P. spicatush* as pharmacological potency and consistency, which could support its use in folklore medicine.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

REFERENCES

Baker CF (1902). *Pseudelephantopus* spicatus (B.juss, ex Aubl). In: Transactions of Academy of Science St. Louis 12(45):55-56.

Bilić-Šobot D, Kubale V, Škrlep M, Čandek-Potokar M, Prevolnik Povše M, Fazarinc G, Škorjanc D (2016). Effect of hydrolysable tannins on intestinal morphology, proliferation and apoptosis in entire male pigs. Archives of Animal Nutrition 70(5):378-388.

Brand-Williams W, Cuvelier M, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissens-chaft-und-Technologie 28:25-30.

Bukhari IA (2013). The central analgesic and anti-inflammatory activities of the methanolic extract of *Carthamus oxycantha*. Journal of Physiology and Pharmacology 64(3):369-375.

Chakraborty P, Bala N, Das S (2018). Analgesic Activity of Methanolic Extract of Tubers of *Arisaema tortuosum* (Wall.) Schott. in Swiss Albino Mice. Dhaka University Journal of Pharmaceutical Sciences 17:37-41.

Chowdhury V, Shristy NT, Rahman MH, Chowdhury TA (2022). Qualitative Phytochemical Screening, Fatty Acid Composition Analysis and Biological Studies of *Trianthema portulacastrum* L. Leaves. Dhaka University Journal of Pharmaceutical Sciences 21(1):33-43.

Heller W, Forkmann G (1994). Biosynthesis of Flavonoids. The flavonoids, advances in research since 1986 (Harborne, J. B., ed.) 11:399-425.

Hasan MM, Taher MA, Rahman MA, Muslim T (2019). Analgesic, Anti-diarrheal, CNS-depressant, Membrane Stabilizing and Cytotoxic Activities of Canavalia virosa (Roxb.) W&A. Bangladesh Pharmaceutical Journal. 22(2):214-218.

Islam M, Jannat T Kuddus, MR (2019). In vitro and in vivo evaluation of pharmacological potentials of *Campsis radicans* LClinical Phytoscience 5:42.

Kupchan SM, Tsou G, Sigel CW (1973). Datiscacin, a novel cytotoxic cucurbitacin 20-acetate from *Datisca glomerata*. Journal of Organic Chemistry 38(7):1420-1421.

Leckie WH (1972). The Negro on the American Frontier by Kenneth Wiggins, Porter Journal of American History 59(1):155.

Maqsood M, Mushtaq Z, Rasheed T, Nisa ZU, Sher F (2021). Thrombolytic and cytotoxic activity of different bioactive extracts of *E. coli*. Case Studies in Chemical and Environmental Engineering 3(100080).

Mekonnen B, Asrie AB, Wubneh ZB (2018). Antidiarrheal activity of 80% methanolic leaf extract of *Justicia schimperiana*. Evidence-Based Complementary and Alternative Medicine 2018:10 pages.

Mohan VR, Chenthurpandy P, Kalidass C (2010) Pharmacognostic and phytochemical investigation of *Elephantopus scaber* L. (Asteraceae). Journal of Pharmaceutical Science and Technology 2(3):191-197.

Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF (2007). Effect of *Fagonia arabica* (Dhamasa) on *in vitro* thrombolysis, BMC Complement Alternat Med 7:7-36.

Quiming N, Minor MR, Nicolas M, Verzosa D, Alvarez MR (2019). Antibacterial Activity of *Pseudoelephantopus spicatus* (Juss) Rohr Extracts from the West Sub-catchment of Mt. Isarog Natural Park. Oriental Journal of Chemistry 35(6).

Rahman A, Hasan MM, Taher MA, Muslim T (2020). Analgesic, Antidiarrheal and CNS-depressant Activities of Flemingia macrophylla (Willd.). Bangladesh Pharmaceutical Journal 23(2):141-145.

Rahman MH, Rashid MA, Chowdhury TA (2019), Studies of Biological Activities of the Roots of *Bombax ceiba* L. Bangladesh Pharmaceutical Journal 22(2):219-223.

Ren Y, Elkington BG, Henkin JM, Sydara K, Kinghorn AD, Soejarto DD (2021). Bioactive small-molecule constituents of lao plants. Journal of Medcinal Plants Research 15(12):540-559.

Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F (2020). Worldwide Research Trends on Medicinal Plants. International Journal of Environmental Research and Public Health 17(10):3376.

Saroar F, Shoeb M, Islam MN, Rhaman MM, Islam R, Parvin N (2020). Studies of marine seaweed *Sargassum flavicans*. Asian Journal of Pharmacognosy. 4:52-58.

Shoba FG, Thomas M (2001). Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. Journal of Ethnopharmacology 76(1) 2001.

Shoeb M (2019). Bioactivity of *Peperomia Pellucida* Leaves from Bangladesh. American Journal of Biomedical Science 6(1):1-3.

Süntar I (2020). Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochemistry Reviews 19(5):1199-1209.

Tsai CC, Lin CC (1999). Anti-inflammatory effects of Taiwan folk medicine 'Teng-Khia-U' on carrageenan- and adjuvant-induced paw edema in rats. Journal of Ethnopharmacology 64(1):85-89.

Velioglu YS, Mazza G, Gao YL and Oomah BD (1988). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agricultural and Food Chemistry 46:4113-4117.