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Exploration of antioxidant, antidiarrheal, analgesic & anti-hyperglycemic potentials and phytochemical screening of ethanolic extract of *Schumannianthus dichotomus* leaves

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Abstract

The Schumannianthus dichotomus plant, known locally as Paitara, is a member of the Marantaceae family. Ethanol was used to extract S. dichotomus leaves. The extract was used to observe the antidiarrheal, antioxidant, anti-hyperglycemic, analgesic, and phytochemical assessment properties. The existence of proteins, alkaloids, terpenoids, glycosides, phenolic compounds, tannins, flavonoids, and saponins were demonstrated by the phytochemical examination of the crude extract of S. dichotomus. The antioxidant assays indicate that the extract has a high total phenolic, tannin, and flavonoid content. Total tannin, phenol and flavonoid in leaves were found 3846.2 mg GAE/100 g, 354.49 mg GAE/100 g (gallic acid equivalent) and 4472.64 mg QE/100 g (quercetin equivalent) respectively. Statistical evaluation does not show any acute toxicity. Thus, it ensures that the negative effects of the crude extract dosage won't influence the pharmacological research's findings. The ethanolic extract was next tested for antidiarrheal, analgesic activity and antidiabetic activity. In the statistical evaluation of antidiabetic activity, the glucose level of four groups were controls 10.3 mmol/L, standard 6.26 mmol/L, extract I 8.3 mmol/L and extract II 12.5 mmol/L. The glucose level was measured by glucometer. In the statistical evaluation of antidiarrheal, the percentage inhibition of defecations standard 65.35%, extract I (250) 45.54%, extract II (500) 52.47%. In this study, loperamide used as standard and also used castor oil which produced diarrhea. Significant analgesic effect was demonstrated by the crude ethanol fraction at 250 mg/kg and 500 mg/kg b.w. of dosages, according to statistical analysis of the data. In contrast, diclofenac sodium (Standard) produced an inhibition of 70.97%, whereas they produced an inhibition of 58.04% and 62.36%, respectively. On the other hand, the leaves of S. dichotomus do not show antibacterial activities. The method is use for this test is called writhing method. The result reveals that the leaves of S. dichotomus exhibit substantial hypoglycemic activity in an ethanolic extract. The findings imply that a variety of phytochemicals with possible antioxidant qualities are present in the extract.

Keywords: Antioxidant, Antidiabetic, Antidiarrheal, Analgesic, Antihyperglycemic, Phytochemical, DPPH

Introduction

Medicine is defined as the science of healing, which includes the practice of diagnosis, health promotion, and illness prevention and treatment ^[1]. It also refers to plant-based compounds, pharmaceuticals, and drugs that are utilized to promote health and treat a range of ailments. According to the medical dictionary, medicine is both a drug and a method of illness prevention. Additionally, it is defined in the investigation and management of general illnesses or conditions that impact the body, especially those that usually do not require surgical intervention ^[2]. It has been demonstrated that one of the plant components is also capable of preventing the onset of certain diseases. By doing this, the adverse effects of synthetic treatment will be lessened, such as the need for chemical therapies when the sickness is already present. A vast number of biologically active compounds with a range of chemical forms that have disease-preventive and curative effects are found in the kingdom of plants (phytochemicals).

Alkaloids, steroids, flavonoids, terpenoids, tannins, and many more phytochemicals are secondary compounds that are frequently found in lesser amounts in higher plants. Only a tiny proportion of medicinally active plants have undergone testing, yet over 50% of pharmaceuticals come from plants. Therefore, the phytochemical analysis of higher plants linked to ethnobotanical knowledge accounts for a significant amount of contemporary study (Harborne et al., 1998). Geographically, the S. dichotomus plant can be found in the Philippines, Peninsular Malaysia, Assam, Burma, Cambodia. Vietnam. Thailand. and northeastern Bangladesh. Traditionally the root is used to cure neuritis (Chiang Mai), Stem used to weave mats and used together with Nypa palm leaves for making roofs, occasionally used for ornamental purposes. Rhizome of the plant is used in fever and stem is used in earache [42]. Md. Mahfuzur Rob et al., 2020 demonstrate the first work to identify phytotoxic compounds from S. dichotomus and report on its phytotoxic potential. The phytotoxic activity may be explained by the growth-inhibiting properties of the compounds found, indicating that its leaves, twigs, or active ingredients may be used as natural bio-herbicides. It has strong analgesic, antidiabetic and antioxidant properties, according to current research. Numerous bioactive substances, including as alkaloids, tannins, flavonoids, saponin, glycoside, proteins, and terpenoids, are revealed by phytochemical tests and lead to the plant's potential for therapeutic application. It is a major topic for more pharmacological research due to its wide range of traditional applications and bioactivities. The examination of the analgesic, antidiarrheal, and antihyperglycemic effects of ethanolic leaf extracts makes this work special. Verifying the plant's traditional usage and investigating its potential for new medicinal applications are the main goals. We intend to advance pharmacognosy and offer a scientific foundation for the use of S. dichotomus in therapies by conducting in vivo research.

Materials and Methods

Collection of plant material: *Schumannianthus dichotomus* leaves were obtained from northeast region of Bangladesh and examined at the Bangladesh National Herbarium.

Extract preparation from plant materials: The leaves were cleansed with water after collection and given a week to dry in the sun. A coarse powder was then made from the leaves using an appropriate grinder. 200 grams of fine powder were put in a clean, glass jar with level bottom and shaken and stirred periodically after being soaked in 1000 milliliters of ethanol for eight days. Usually, a piece of fresh, white cotton was used to remove the whole concoction. The crude extract was later discovered through filtration utilizing Whatman filter paper.

Qualitative phytochemical screening: The extract has gone through several standard procedures like Dragendorff's test, Molish's test, Benedict's test, Fehling's test, Alkaline test, Xanthoproteic test, Ferric chloride test, Potassium dichromate test, Sulfuric acid test, Libermann-Burchard test, Froth test, and Sodium bicarbonate test to determine different chemical groups (Trease & Evans, 1989; Sofowara, 1982). For each test, an extract solution in ethanol at 5% (w/v) was used.

In vitro assays

Assessment of antioxidant potentials

- Total tannin content: The total tannin content of the leaf extract was determined using analytical grade gallic acid as the standard and the Folin-Ciocalteu (FC) reagent. 0.5 ml of FC reagent and 7.5 ml of distilled were added following solutions concentrations ranging from 20 to 0.1 milliliters of gallic acid solution were used to create 100 mg/L. Ten milliliters of distilled water were used to dilute one milliliter of a 35% sodium carbonate solution that had been added five minutes earlier. After 30 minutes, the UV absorbance at 725 nm was precisely measured against the blank. With the exception of adding sample and gallic acid, the same procedures were used to make the blank. The standard curve was used to calculate the extract's total tannin content, which was then represented as mg Gallic acid equivalent (GAE)/100 g dried plant extract.
- Total flavonoid content: The total flavonoid content of the crude extract was ascertained using the Meda *et al.* (2005) technique. Quercetin served as the standard in this particular case. This procedure involved adding 4 ml of distilled water while stirring 1 ml of quercetin solution at each concentration (20-80 µg/L) in a 10 ml volumetric flask. Next, two milliliters of 1 M sodium hydroxide, 0.3 milliliters of 5% sodium nitrous solution, and 0.3 milliliters of 10% aluminum chloride were added, respectively. After adjusting the final volume to 10 ml, each concentration's UV absorbance at 510 nm is measured against a blank. The standard curve was used to assess the extract's total tannin content, which was stated as milligrams of quercetin equivalent (QE) per 100 grams of dried plant extract.
- Total phenolic content: The total phenolic content of the ethanol extract of S. dichotomus was measured in this study using the Folin-Ciocalteu (FC) reagent, with analytical grade Gallic acid serving as the standard. (Marinova et al., 2005). One milliliter of extract or standard solution (15.62-500 mg/L) was combined with 9 ml of distilled water. After being diluted for ten times with distilled water, one milliliter of FC reagent was added. Ten milliliters of 7% Na₂CO₃ were added to the mixture after five minutes. It is then allowed to sit at temperature for 30 minutes. Α room spectrophotometer was then used to measure absorbance at 750 nm in relation to a blank. The extract's total phenolic content was evaluated utilizing the standard curve and stated as mg Gallic acid equivalent (GAE)/100 g dried plant extract.

In vivo assays

Animals used in research: The *in vivo* tests were performed adopting Swiss albino mice obtained from Jahangirnagar University. The animals had free access to food and water and were housed in cages at room temperature with a 12-hour light-dark cycle. Consent and ethical clearance were granted by the Committee of Clinical Pharmacy & Pharmacology, Department of Pharmacy and Dhaka International University.

Evaluation of anti-hyperglycemic activity

Oral glucose tolerance method: With a few minor adjustments, oral glucose tolerance tests (OGTT) were

conducted in accordance with Joy and Kuttan's (1999) protocol. The test animals were fasting, meaning they were only given water and no food or liquids for at least 10 but no more than 16 hours. Five mice were split up into four groups (N=5), which were designated Group I, Group II, Group III, and Group IV. Each group received a specific treatment, including control, standard, and test samples. The standard (glibenclamide) and control (1% Tween 80 solution in water) were administered orally at zero-hour test samples at doses of 250 mg/kg and 500 mg/kg using a feeding needle. After half an hour, each group received a 10% glucose solution (2 gm/kg body weight). After 60, 90, and 150 minutes, blood samples drawn from the tail vein were utilized to measure the blood glucose level using a glucometer.

Anti-diarrheal activity evaluation

Castor oil-induced diarrheal method: The castor oilinduced diarrheal technique was used to assess the antidiarrheal activity. Group I (control group), Group II (standard group), Group III (test sample-1), and Group IV (test sample-2) were the four groups (N=5) into which the experimental animals were randomly assigned. The control group received distilled water with 1% Tween-80, whereas the standard group received loperamide at a dosage of 3 mg/kg body weight. A suspension of S. dichotomus leaf extract was administered orally to Group III and Group IV at 250 mg/kg and 500 mg/kg body weight, respectively. One hour before to the oral delivery of 0.5 milliliters of castor oil per mouse, the test animals given the treatment. For four hours, each mouse was kept in a different hutch with blotting sheets in each cage to check for the occurrence of diarrhea. Over 4 hours, stool and fluid material staining were counted, and new papers were placed at each hour.

Evaluation of Analgesic Activity

Acetic acid-induced writhing reflex method: The analgesic impacts of *S. dichotomus* extract were evaluated using mice's writhing upon encountering acetic acid, as reported by ^[47-50]. Group I, Group II, Group III, and Group IV are the names of the four groups (N=5) into which the experimental animals were randomly assigned. Each group was given a specific treatment, which included two doses of

the extract (250 mg/kg and 500 mg/kg body weight), a positive control (diclofenac sodium), and a negative control (1% Tween-80 solution in water). Using a feeding needle, test samples as well as positive and negative control solutions were administered orallyFor optimal absorption, a 30-minute break was provided. A 0.7% acetic acid solution was then injected subsequently into all the mice in the group, causing them to writhe. After five minutes, the number of writhing squirms was counted for fifteen minutes.

Statistical analysis

For both *in vitro* and *in vivo* tests, MS Excel (version 2010) was used to support statistically significant results.

Results

Phytochemical screening: In the qualitative study of phytochemical screening, the *S. dichotomus* leaf extort demonstrated an abundance of proteins, alkaloids, tannins, flavonoids, terpenoids, glycosides, and saponins (Table 1). These Phyto compounds may be the root cause of the leaf's pharmacological potential ^[55].

Table 1: The finding of the *Schumannianthus dichotomus* chemical group test

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⁺ indicates Presence and-indicates Absence

Hypoglycemic Activity

Test Effects of *S. dichotomus* on blood glucose treated diabetic mice are shown in the Table 2, Figure 1, 2 and (3).

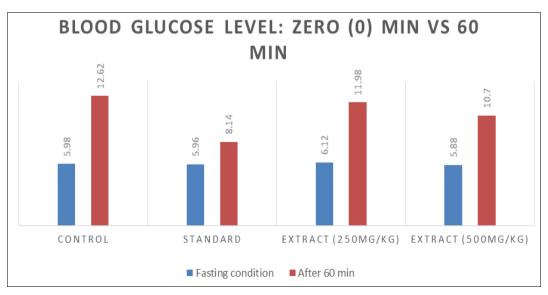


Fig 1: Blood Glucose Level (mg/dL): Zero (0) min Vs 60 min

Table 2: Blood Glucose Level in various time and condition (Avg) with significant value: (Avg ± SEM-Average Glucose Level ± Standard Error Mean).

Blood Glucose Level in various time and condition (Avg)							
Group (Dose)	Fasting condition	After feeding glucose					
	$(Avg \pm SEM)$	After 60 min	After 90 min	After 150 min			
Control	5.98±0.26	12.62±0.27	11.06±0.36	10.3±1.06			
Standard	5.96±0.18	8.14±0.37	6.76±0.28	6.26±0.41			
Extract (250mg/kg)	6.12±0.17	11.98±0.40	10.72±0.58	8.3±1.24			
Extract (500mg/kg)	5.88±0.32	10.7±0.44	11.3±0.70	12.5±0.37			

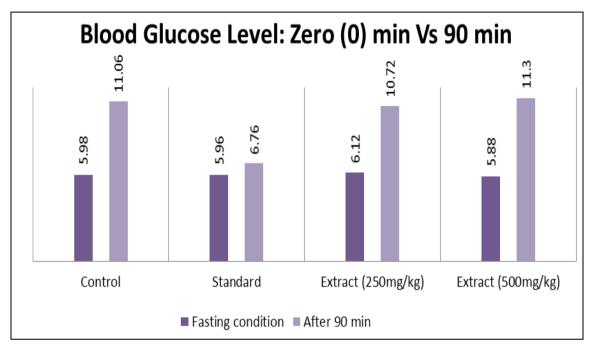


Fig 2: Blood Glucose Level: Zero (0) min Vs 90 min

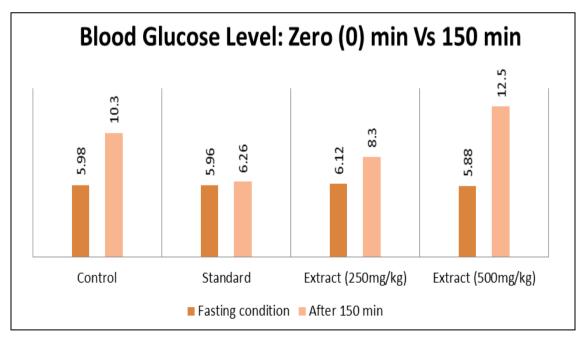


Fig 3: Blood Glucose Level: Zero (0) min Vs 150 min

Assessment of in vivo antidiarrheal activity

Table 3: Impact of S. dichotomus leaf extract on extending the undetected duration of a diarrheal episode in mice produced by castor oil

Group	Number of mice	Latent period (min)	Mean latent period (min)	SD	SEM	T-Test (P-Value)
	C ₁	34				
I (Control)	C_2	29				
1% tween-80 in water	C ₃	31	30.8	2.39	1.07	
1 % tween-80 m water	C_4	28				
	C ₅	32				
	S_1	175				
П	S_2	127				7.9968 (p<0.0001)
(Standard) Loperamide 3 mg/kg	S ₃	195	149.6	33.13	14.42	Significant
(Standard) Loperannue 5 mg/kg	S ₄	128				
	S ₅	123				
	T_1	77		13.94	6.23	5.7870 (p<0.0004) Significant
III (Test group) Extract	T_2	65	67.4			
250 mg/kg	T ₃	55	07.4			
250 mg/kg	T ₄	86				
	T ₅	54				
	T_1	124				
IV (Test enough) Extract	T_2	134				0.2706 (= <0.0001)
IV (Test group) Extract 500 mg/kg	T ₃	155	124.6	22.26	9.95	9.3706 (p<0.0001) Significant
Joo nig/kg	T_4	115]			Significant
	T ₅	95				

SD-Standard deviation, SEM-Standard Error for Mean

Table 4: Impact of S. dichotomus leaf extract on stool count during a diarrheal episode in mice caused by castor oil

Group	Number of mice	No. of stool after 4 h.	Mean no of stool	% inhibition of defecation	SD	SEM	T-Test (P-value)
	C_1	19					
I (Control)	C_2	22			2.39		
1% tween-80 in water	C ₃	17	20.2			1.07	
170 tween-00 iii water	C ₄	23					
	C ₅	20					
	S_1	12					7.4492
II (Standard) Loperamide	S_2	8		65.35	3.16	1.41	(p<0.0001)
3 mg/kg	S_3	6	7				Significant
3 mg/kg	S_4	5					
	S_5	4					
	T_1	11		45.54	3.81	1.70	4.5772
III (Test group) Extract	T_2	16					(p<0.0012)
250 mg/kg	T ₃	13	11				Significant
250 Hig/kg	T_4	9					
	T ₅	8					
	T_1	6					
IV (Tost group) Extract	T_2	8				1.17	6.7040
IV (Test group) Extract 500 mg/kg	T ₃	12	9.6	52.47	2.61		(p<0.0002)
Joo nig/kg	T_4	10					Significant
	T ₅	12					

SD-Standard deviation, SEM-Standard Error Mean

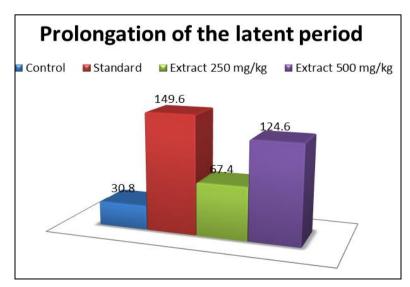


Fig 4: The impact of S. dichotomus leaf extract on extending the latent period in mice's diarrheal episodes caused by castor oil

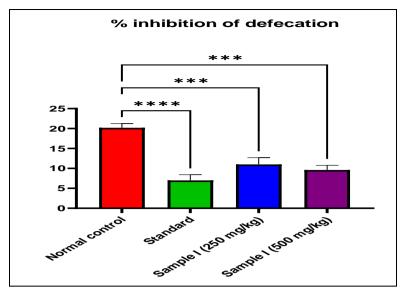


Fig 5: Impact of leaf extract based on the% reduction in feces in mice with diarrheal episodes produced by castor oil

Evaluation of Analgesic Activity

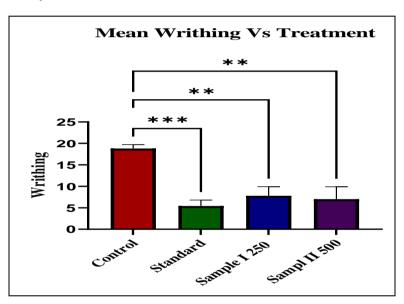


Fig 6: Percent (%) inhibition of writhing vs. treatment

Table 5: Effects of S. dichotomus extract on acetic acid induced writhing of the test animals

Dosage given to the test animals	No of mice	Weight (gm) of the mice	Writhing of mice	Average Writhing
	1	24	17	
	2	22	21	
Control	3	26	19	18.6
	4	28	16	
	5	23	20	
	1	23	2	
	2	26	10	
Positive Control Diclofenac Na (25 mg/kg)	3	25	6	5.4
	4	20	3	
	5	26	6	
	1	24	8	
	2	24	6	
ES (250mg/kg)	3	25	2	7.8
	4	22	8	
	5	24	15	
	1	22	3	
	2	22	15	
ES (500mg/kg)	3	25	1	7
	4	23	13	
	5	21	3	

Table 6: Statistical assessments of impacts of the extracts on acetic acid induced writhing

Animal group	Mean of Writhing ± SEM	% Writhing	% Inhibition of writhing	T-Test (value of p)
Negative control	18.6 ± 2.07	100		
Positive Control Diclofenac Na (25 mg/kg)	5.4 ± 1.40 ***	29.03	70.97	7.8605 (p<0.0001) Significant
ES (250 mg/kg)	7.8 ± 2.11**	41.93	58.06	4.6912 (p<0.0016) Significant
ES (500 mg/kg)	7 ± 2.90**	37.63	62.36	3.8120 (p<0.0051) Significant

Significance: * p<0.05, ** p<0.01, ***p<0.001

Determination of total tannin content

Table 7: Gallic acid (standard) UV absorbance at 725 nm

Concentration (mg/L)	Average	Absorbance I	Absorbance II
20	0.0285	0.028	0.029
40	0.0435	0.043	0.044
60	0.0555	0.057	0.054
80	0.061	0.062	0.06
100	0.073	0.074	0.072

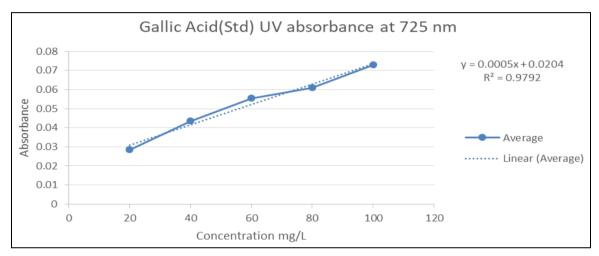


Fig 7: Gallic Acid (STD) UV absorbance at 725 nm

The total tannin content of S. dichotomus leaf extract showed to be 3846.2 mg GAE/100 g of dried plant extract.

Estimation the total amount of flavonoids

Table 8: Quercetin (standard) UV Absorbance at 510 nm

Concentration (µg/L)	Average	Absorbance I	Absorbance II
20	0.011	0.012	0.01
40	0.0275	0.027	0.028
60	0.036	0.038	0.034
80	0.0445	0.044	0.045
100	0.068	0.069	0.067

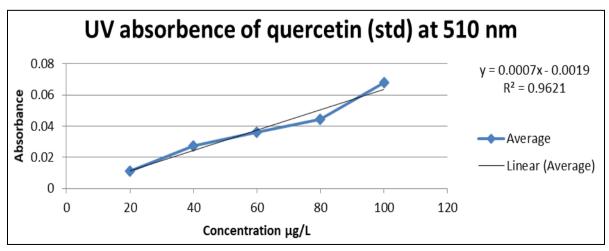


Fig 8: UV absorbance of quercetin (STD) at 510 nm

The total flavonoid materials of extract exposed to be 4472.64 mg QE/100 g of extracts from dried plants.

Estimation of the whole phenolic material

Table 9: Gallic acid's (standard) UV absorbance at 750 nm

Concentration (mg/L)	Average	Absorbance I	Absorbance II	Absorbance III
15.62	0.07833333	0.084	0.076	0.075
31.25	0.129	0.115	0.136	0.136
62.5	0.26533333	0.263	0.266	0.267
125	1.19266667	1.19	1.194	1.194
250	1.68833333	1.691	1.686	1.688
500	2.88266667	2.882	2.885	2.881

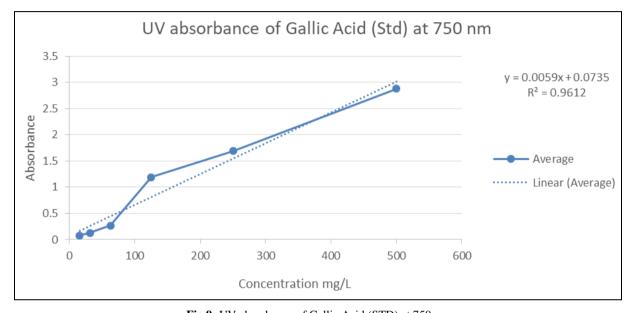


Fig 9: UV absorbance of Gallic Acid (STD) at 750 nm

No	1st Rea	ding	2 nd Reading	Average	SEM	Grand Average
1	2.15	8	2.172	2.165		
2	2.16	7	2.166	2.1665	0.001061	2.165 ± 0.001061
				2.16575		
	X		у		m	c
354.4	1915254		2.165		0.0059	0.0735

The leaf extract *of S. dichotomus* was found to have a total amount of phenolic substances of 354.49 mg GAE/100 g of dried plant extract.

Discussions:

For many years, Schumannianthus dichotomus has been utilized in traditional medicine. There are many studies on its bark, root, and rhizome, but less study on its leaves. This study was designed to fulfill the evaluations of phytochemical group test, anti-diarrheal, analgesic, antidiabetic, antioxidant properties of the extract of ethanol of S. dichotomus leaves. The plant sample being tested contains alkaloids, tannins, flavonoid, carbohydrate, glycosides, protein and saponin as indicated by the plus sign in the result column. At three exceptional time points (60, 90, and 150 minutes), the blood glucose levels of nondiabetic and diabetes-prone mice were measured under four different conditions: control, standard, elicit 250 mg/kg, and elicit 500 mg/kg of S. dichotomus ethanolic extract. Normal control diabetic mice showed a change in blood glucose levels to 5.98 mmol/L while fasting. The control group's glucose level was then elevated at 12.62 mmol/L after 60 minutes. On the other hand, the glucose level of standard,

elicit I (250 mg/kg) and elicit II (500 mg/kg) was comparatively low than control group which are 8.14mmo/L, 11.98 mmol/L and 10.7 mmol/L. The glucose level in the control group was 11.06 mmol/L at 60 minutes, and in the extract 250 mg/kg group, it was 10.72 mmol/L. However, we can observe a significant change in the standard group at 90 minutes, which was 6.76 mmol/L. Additionally, we see that extract 500 mg/kg is rather higher than 60 min. In the last observation at 150 min the lowest glucose level of standard group which is 6.26 mmol/L and the highest glucose level of group extract group II (500mg/kg) which is 12.5 mmol/L. So, we can see except extract 500 mg/kg group standard and extract I (250 mg/kg) who able to lowing glucose level in mice body. The glucose level of four group controls 10.3 mmol/L, standard 6.26 mmol/L, extract I 8.3 mmol/L and extract II is 12.5 mmol/L. At amount of 250 and 500 mg/kg b. w., the ethanolic leaf extract considerably (p<0.0001) lessened the entire quantity of stools and postponed the onset of diarrhea in the castor oil-induced diarrheal mice. At quantity of 250 and 500 mg/kg b. w., the percentage of defecation inhibition was 45.54 and 52.47, respectively. At amount of 3 mg/kg b. w., standard loperamide similarly demonstrated a reduction in the entire quantity of stools and a 65.35% suppression of defecation. According to the test results, the standard medication Diclofenac Na inhibited the writhing reflex by 70.97% at a dose of 25 mg/kg body weight, whereas the leaves extract inhibited it by 58.06% and 62.36% at amount of 250 mg/kg and 500 mg/kg, reverentially. Every value has been contrasted with that of the negative control group.

Additionally, the extract from *S. dichotomus* leaves produced a statistically significant finding. The findings of the statistical analysis are as follows: Significant differences were seen between the negative control and Diclofenac Sodium, ES (250 mg/kg), and ES (500 mg/kg). Therefore, while the criterion for statistical consequence was established at 0.05, it could be said that the extract's analgesic efficacy was significant when compared to negative control animals. Leaf extract was obtained to have a total tannin, flavonoid, and phenolic substance of 3846.2 mg GAE/100 g, 4472.64 mg QE/100 g, and 354.49 mg GAE/100 g of dried plant elicit, respectively.

Conclusion

This study is an emerging field of research in evaluating many pharmacological effects. If the proposed research can be performed successfully, a valuable resource for potent drug can be obtained. That may provide a safe, effective, economic and convenient source of potential drugs with desirable clinical properties. If we get opportunity, we will carry on our research to evaluate other pharmacological properties. Leaves of S. dichotomus were macerated in 70% ethanol to produce extract which was tested for a number of in vivo and in vitro biological research e.g. hypoglycemic activity, analgesic activity, antibacterial, antidiarrheal activity and antioxidant activity (total phenolic, tannins and flavonoid material). Different concentration of this extract was statistically evaluated and significant activity was seen for the in vivo tests except antibacterial test. Test groups were significantly different for more time points from control as the concentration increased. Only higher concentration of extracts was significantly exerting these activities. In case of in vitro tests, extract showed very low cytotoxicity which would very much fall in line with the traditional use of S. dichotomus. The extract presented mild anti diabetic activity and also it does not show any antibacterial activities but all other activities were good as compared to standards. Therefore, leaves of S. dichotomus can be used to prepare natural products which can be safely taken by all people. Molecular mechanism of the biological activities can also be determined for ensuring individual treatment efficiency, efficacy and compliance. This study offers valuable information regarding the biological activity and composition of the plant extracts, and it could be a foundation for future investigations into the creation of novel CNS depression and antioxidant medicines Based on the results mentioned above, it can be said that the plant extract can control hyperglycemia, lessen pain and keep diarrhea under control.

List of Abbreviations

DPPH: 2,2-Diphenyl-1-picrylhydrazyl, CE: Crude extract, SD: Standard deviation; SEM: Standard error mean

Conflicts of Interest

The study was carried out without any financial or commercial ties that might be interpreted as a conflict of interest, according to the authors.

Author Contributions Statement

Conception & Supervision: Shahenul Islam; Data curation: Md. Hamedul Islam, Sohag Molla, Sabrina Sultana, Mohammad Mimkul Islam; Formal analysis: Shahenul Islam, Tasnim Jahan, Sohag Molla; Investigation: Shahenul

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Statement of Data Availability

Data pertinent to the investigation is attached in the supplements. Upon contacting the appropriate person, raw data will be sent upon reasonable request.

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