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Evaluation of Analgesic, Antidiarrheal and Hypoglycemic Activities of *Wendlandia paniculata* (Roxb.) DC Leaves Extract using Mice Model

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Abstract

The leaves of *Wendlandia paniculata* (Roxb.) DC is a rare folk medicine among tribal people even though there is no scientific literature available regarding its pharmacological potentials. This study aimed to evaluate the effects of crude methanol extract of *W. paniculata* (Roxb.) DC. Leaves in mice model. The *in vivo* glucose-lowering capacity of methanolic crude extract was analyzed utilizing the well-known tail tipping method. The central and peripheral analgesic activity was evaluated using the 'tail flick' and writhing assay respectively. The anti-diarrheal activity was evaluated using the method of castor oil-induced diarrhea in mice. As a result, oral administration (400 mg/kg) of the extract resulted significant ($P<0.001$) delay in response to pain and inhibition of acetic acid-induced writhing response. The results were comparable to the respective standards morphine (2 mg/kg) and diclofenac (50 mg/kg). Likewise, maximum reduction ($p<0.05$) of blood glucose (32.3%) was observed 180 min after oral intake(400 mg/kg) of the extract compared to the 47% of the standard glibenclamide. Moreover, a reduction of diarrheal feces was observed 45.83% ($p<0.001$) compared to standard loperamide 70.83%. The *in vivo* bioassays confirmed that the crude methanol extract of leaves of *W. paniculata* possesses significant analgesic (central, peripheral) and antidiarrheal activity with mild glucose-lowering activity. Isolation of bioactive compounds is needed to justify the molecule responsible for the activity.

Keywords: Analgesic, Antidiarrheal, Hypoglycemic, *Wendlandia paniculata*

1. Introduction

Medicinal properties of plants were known even to pre-memorable men and a large number of these plants have been utilized in conventional medication for a long time with notoriety as an adequate cure[1]. Phytotherapy is by all accounts a substitute arrangement of medication for the individuals dwelling in the rural/country territories[2]. As indicated by the WHO, almost 80% of the total populace depends on customary medication for their essential health care[3]. In the course of the only remaining century, ethnobotany has developed into a particular control that takes a gander at the individual's plant relationship in a multidisciplinary path, for example, environment, financial organic science, pharmacology and public healthscience[4]. An enormous number of plants are being utilized as medicinal operators everywhere throughout the world. 1500 species in India[5], 5000 species in China and 1600 species in north-westAmazonia[6] have been accounted for to have restorative utilization. The lack of

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synthetic products and the cost-effectiveness of herbal medicine have made medicinal plants valuable day by day[7]. Many medications that are as of now in the market have originated from folk medication and conventional utilization of plants by the indigenous community[8] Revealing the cardiac activity of the leaves of *Digitalis purpurea* that were valuable for treating dropsy is the best case of people utilize based homegrown medication[9]. About 25% of the doctor prescribed medications gave in the USA and Canada contain a bioactive molecule that is prepared from natural products[10].

Wendlandia paniculata (Roxb.) DC is a very rare plant in Bangladesh that is used by the Chakmas tribe in hill tracts areas in Bangladesh. It has been used as a remedy for chest pain by rubbing the crushed leaves on the chest[11]. Wendland is a genus of flowering plants in the family Rubiaceae. It is found in northeastern tropical Africa, and from tropical and subtropical Asia to Queensland.

Diabetes mellitus is brought about by the variation from the norm of sugar digestion which is connected to low blood insulin level or lack of sensitivity of target organs to insulin[12]. The natural products medications with anti-diabetic properties are yet to be economically planned as present-day prescriptions, even though they have been acclaimed for their remedial properties in the conventional frameworks of medication.[13] The plants give an expected wellspring of hypoglycemic medications in light of the fact that numerous plants and plant inferred compounds have been utilized in the treatment of diabetes. Hypoglycemic herbs increment insulin emission, upgrade glucose take-up by fat or muscle tissues and restrain glucose absorption from the digestive system and glucose creation from the liver[14]. Pain derives in numerous structures acute, chronic, visceral, inflammatory, or neuropathic[15,16] and ¼th of Americans, for instance, experience pain consistently. Having the numbers on its side, the pain turned into a worldwide general medical issue and the main source of handicap everywhere throughout the world[17]. The medicinal utilization of plants as pain-relieving drugs in rural medication is an old convention[18,19]. The investigation for new pain-relieving bioactive compounds from the medicinal plant is growing. Looseness of the bowels can be characterized as a gastrointestinal issue where there is a quick travel of gastric substance through the digestive system, which is portrayed by unusual smoothness and high recurrence of fecal departure, normally semisolid or watery fecal issue, at least multiple times/day[20–22]. The major causative operators of loose bowels in people incorporate an assortment of enteric pathogenic microorganisms, for example, *Salmonella typhi, Shigella flexneri, Escherichia coli, Staphylococcus aureus, Vibrio cholerae,* and *Candida albicans*[23,24]. Flavonoids and saponins hinder the arrival of prostaglandins, autocoids, and compressions brought about by spasmogens just as motility and hydroelectrolytic emissions[25,26] while saponins may forestall the arrival of histamine[27]. Polyphenols and tannins give solidarity to the intestinal mucosa, decline intestinal discharge, intestinal travel and advance balance in water transport over the mucosal cells[28].

A rigorous literature search demonstrates that there is no scientific report on the biological examinations of the plant to affirm its medicinal properties. Thusly, in this examination, the plant *W. paniculata* (Roxb.) was completely examined for assessment of pharmacological possibilities by in vivo analgesic, hypoglycemic, anti-diarrheal activities in a suitable experimental model.

2. Material and Methods

2.1 Collection of Plant Materials

The leaves of *W. paniculata* (Roxb.) were gathered from hill tracts of Chittagong, Bangladesh, sun-dried for a 20 days,and afterward, stove dried for 25 hours at impressively low temperature (not more than 40^0C). The dried natural products were then ground into coarse powder utilizing high-limit granulating machine in the Phytochemical Research Laboratory, State University of Bangladesh. The powdered material (300gm) was taken in a cleaned, golden shading reagent bottle (2500 ml) and absorbed 2000 ml of methanol. The holder with its substance was fixed by bottle top and saved for a time of 15 days going with infrequent shaking and blending. The entire blends were then sifted through a new cotton plug lastly with a WhatmanNo.1 channel paper. The volume of the filtrate was then permitted to vanish at the surrounding temperature until around 70% dissolvable was dissipated[29].

2.2 Animals

Swiss Albino mice (male) weighing between 25–35 g and 4–5 weeks old were gotten from Jahangirnagar University. The mice were kept in the animal house of the State University of Bangladesh and took care of with standard rat feed under carefully looked after conditions. Natural changes were deliberately observed and preceding any test, the creatures were permitted (4 days) to conform to the new ecological conditions. The Federation of European Laboratory Animal Science Associations (FELASA) rules and proposals were followed to lessen the agony and worry of the exploratory mice. For the in vivo bioassays, the creatures were isolated into four groups (Group I,II,III and IV) of 3 animals in

each group. The initial two clusters (I and II) filled in as the negative and positive control, while group III and took care of with 200 and 400 mg for each kilogram body weight (p.o) of unrefined concentrate[30].

2.3 Drugs and Chemicals

Glibenclamide, loperamide, acetylsalicylic acid and diclofenac sodium were the products of Beximco Pharmaceutical Ltd., Bangladesh. Morphine injections were taken from the Ganashastha hospital, Dhanmondi. All the reagents were ensured to be of analytical grade.

2.4 Data Analysis

All the results were recorded in triplicates and results expressed as the mean ± standard error mean (SEM). In order to determine the significance in variables between groups, One way ANOVA and Post Hoc Dunnett's test were performed using SPSS version 25.0 and p-value less than 0.05 was considered to be statistically significant.

2.5 Hypoglycemic Activity

The in vivo glucose-lowering capacity of methanolic crude extract was analyzed utilizing the well-known tail tipping method[31]. After a short-term fasting period, four groups of (n = 3) were treated with 200 and 400 mg of methanolic crude extract orally, 5 mg glibenclamide, 1% Tween-80 with saline solution, per kg body weight individually. 60 min later 10% glucose solution (2 g/kg b.wt.) were given orally to all the mice and blood glucose level were recorded over 180 min.

2.6 Evaluation of Peripheral and Central analgesic Activity

The analgesic effect was addressed by utilizing the tail immersion (central) and writhing (peripheral) method proposed by Pizziketti, *et al.,* 1985 and Kaushik, *et al.,* 2012 respectively[32,33]. In the first test response time to heat was recorded over a period of 90 min, after the oral administration of *W. paniculata* crude extract and using morphine as the standard. Whereas, the second experiment recorded the inhibition of salicylic acid-induced 'writhing' response in mice for a duration of 4 hr and diclofenac sodium was considered as the reference standard.

2.7 Anti-diarrheal Activity by Castor Oil Challenge

The anti-diarrheal activity of the methanolic extract of leaves of *W. paniculata* Roxb. was evaluated using the method of castor oil-induced diarrhea in mice (Shoba & Thomas, 2001). According to this method[34], each mouse was fed with 1ml of the highly pure analytical grade of castor oil which would induce diarrhea. The numbers of fecal stools were recorded for each mouse. The observations of the experimental groups were compared against that of the control to evaluate the anti-diarrheal activity of the samples.

3. Results

3.1 Anti-hyperglycemic Activity of *W. paniculata* Crude Extract

The *W. paniculata* crude extract of 400 mg/kg.b.wt., started to show significant ($p<0.05$) effect 180 min. after oral administration while the standard glibenclamide initiated its effect ($p<0.05$) 60 min. prior to the crude extract and continued for the whole 3 hr. The SSCE at 400 mg/kg. resulted in a maximum of 32.3% reduction ($p<0.001$) of blood glucose 180 min after the oral intake while glibenclamide (5 mg/kg) reduced blood glucose by 70% ($p<0.001$) at the same time (Table 1).

Table 1. Effect of *W. paniculata* crude extract on the glucose induced hyperglycemia in Swiss albino mice

Test groups (mg/kg)	n	Average blood glucose level (mmol/l)					% Inhibition	
		Before treatment	After treatment					
		0 min	30 min	60 min	120 min	180 min	120 min	180 min
Control	3	4.9±0.31	16.5±1.73	12.7±1.5	9.97±1.3	6.2±1.01		
Standard (5)	3	3.9±0.31	15.8±0.55	7.9±0.5*	3.9±0.25**	1.87±0.2 **	61%	70%
MESF (200)	3	4.5±0.56	16.9±1.39	12.6±1.5	9.9±1.38	5.3±0.76	1%	15%
MESF (400)	3	4.73±0.46	17.2±2.7	10.8±0.4	8.17±0.38	4.2±0.55*	18%	32.30%

3.2 Central and Peripheral Analgesic Activity of *W. paniculata* Crude Extract

Both the *W. paniculata* crude extract doses and morphine showed their first sign of action on the 30 min mark and continuing for a total 90 min hence the duration of the experiment. The *W. paniculata* crude extract at a dose of 200 mg/kg (p.o.) elongated the response time (P<0.001) to heat by 135.80 % (30 min) and 340.13 % (90 min) in the end (Table 2).

Table 2. Effect of *W. paniculata* crude fruit extract in the tail-flick assay in Swiss albino mice

Test groups (mg/kg)	Average blood glucose level (mmol/l)				% Elongation			
	0 min	30 min	60 min	90 min	0 min	30 min	60 min	90 min
Control (1% Tween 80)	1.49±0.04	2.38±0.12	2.09±0.14	2.23±0.03				
Morphine (2 mg/kg)	1.69±0.09	5±0.27***	9.02±0.22***	13.96±0.13***	13.75%	236.25%	506.50%	838.79%
MESF (200mg/kg)	1.98±0.18	3.51±0.15**	4.96±0.08****	6.54±0.2***	32.88%	135.80%	233.78%	340.13%
MESF (400mg/kg)	1.99±0.17	3.52±0.02**	5.3±0.12*****	7.52±0.09****	33.78%	136.85%	256.35%	405.53%

Here, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001when compared with control group. SEM = Standard error of mean, WPCE = *W. paniculata* crude extract, n = Sample size. Whereas the morphine inhibited (P<0.001) the response time by 236.25 % (30 min) which increased up to 838.79% 1.5 hr after administration. The *W. paniculata* crude extract also exhibited significant peripheral analgesia comparable to the standard diclofenac sodium (Table 3).

Table 3. Effect of *W. Paniculata* crude fruit extract in the ASA induced writhing test in Swiss albino mice

Test groups	Writhing count			Number of writhing Mean ± SEM	% writhing	% Inhibition of writhing
	M1	M2	M3			
Control (1% tween-80)	20	22	19	20.33±0.88	100	-
Diclofenac sodium (Std.) (50 mg/kg)	3	4	3	****3.33±0.33	16	84
MESF (200 mg/kg)	15	12	11	***12.67±1.2	62	38
MESF (400 mg/kg)	8	7	9	5.67±0.33	39	64.58

Here, *p < 0.05, **p < 0.01 and ***p < 0.001 and ****p < 0.0001 when compared with control group. SEM = Standard error of mean, WPCE = *W. Paniculata* crude extract, n = Sample size.

The extract at 400 mg per kg inhibited salicylic acid induced writhing response by 64.58 % (P<0.001) while 84% reduction (p<0.001) was recorded for the standard (Figure 1. a, b).

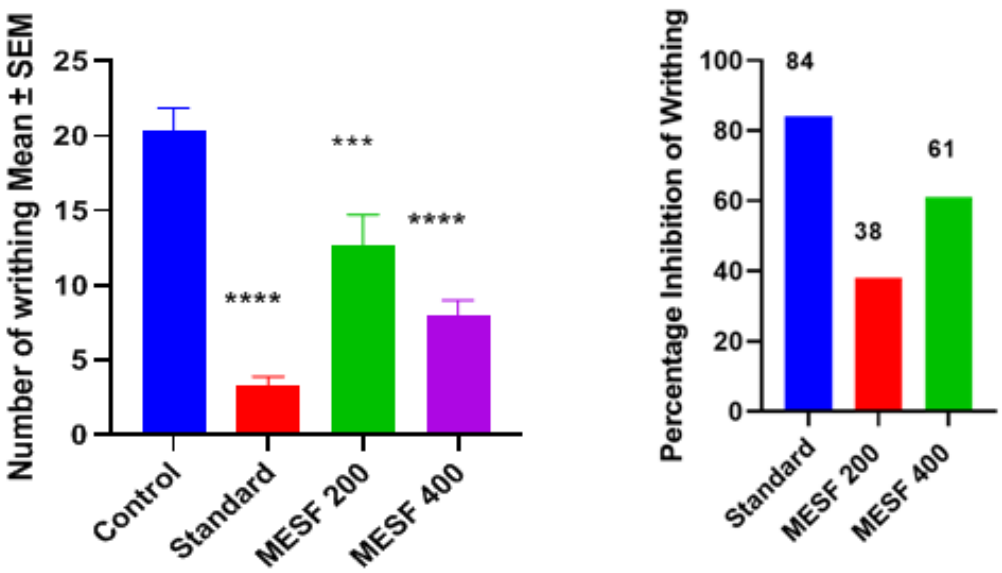


Figure 1. Effect of the oral administration of the WPCE on the ASA induced writhing test in Swiss albino mice. Here, all the values are expressed as mean ± SEM and SEM = Standard error of mean, WPCE = *W. paniculata* crude fruit extract. (*) indicates $p < 0.05$, (**) indicates $p<0.01$ and (***) indicates $p<0.001$ and ****$p < 0.0001$ compared to the normal group.

3.3 Antidiarrheal Activity of *W. paniculata* Crude Extract

The crude methanol extract of *W. paniculata* exhibited a significant reduction of average diarrheal feces at a dose of 400 mg/kg ($p < 0.0001$) and the results were comparable to the standard antidiarrheal drug loperamide (2 mg/kg orally). 4 hours after the oral treatment the number of diarrheal feces was found to be 8±0, 2.33±0.33, 5 ±0.577, 4.33±0.33, in the control, positive control and the groups receiving the 200mg and 400mg of the plant extract respectively (Table 4).

Table 4. Effect of *W. Paniculata* crude fruit extract in the castor oil induced antidiarrheal test in Swiss albino mice

Test Groups	n	Number of diarrheal feces Mean±SEM	% Reduction of diarrheal feces
Control (1% tween-80)	3	8±0	-
Loperamide (std.) (2 mg/kg.b.wt)	3	2.33±0.33****	70.83
MESF (200 mg/kg.b.wt)	3	5±0.577**	37.5
MESF (400 mg/kg.b.wt)	3	4.33±0.33***	45.83

Here, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ and ****$p < 0.0001$ when compared with control group. SEM = Standard error of mean, WPCE = *W. Paniculata* crude extract, n = Sample size

4. Discussions

The methanol extract of *W. paniculata* demonstrated a moderate reduction of blood glucose after glucose-induced hyperglycemia in swiss albino mice. Increased production of insulin by the β-cells in the pancreas and expanded take-up of glucose by liver and muscle tissues are the two basic phenomena that could decrease sugar fixation in the blood[35]. Phytoconstituents, for example, alkaloids are characteristically hypoglycemic in nature while flavonoids have been found to upgrade peripheral glucose take-up and cell glycolysis[36,37]. Also, saponins were found to animate pancreatic β-cells in this way expanding insulin fixation in the blood[38]. Different heterocyclic compounds like indole, alkaloids (isocodonocarpine), flavonoids and triterpenoids have been accounted for to be found richly in the different types of Rubiaceae family. The extract could induce an increment in glucokinase activity in diabetic rats, which in turn regulated glycogen metabolism[39]. Hence the hypoglycemic action of the *W. paniculata* could be ascribed due to the nearness of these phytochemicals.

The 'tail flick' strategy is a typical technique broadly utilized for considering impacts on the nociceptive pain in mice model. A huge reaction to this bioassay shows the nearness of phytochemical agents' capable actuating analgesic activity similar to morphine[40]. On the other hand the acetic acid-induced 'writhing' response for determining peripheral analgesia has been associated with the release of prostaglandins in the periphery via COX pathway[41]. The crude concentrate of *W. paniculata* was found to significantly ($p<0.001$) diminish the writhing response and therefore might contain phytochemicals interfering with the peripheral prostaglandin synthesis pathway[42]. (Figure 2. a, b).

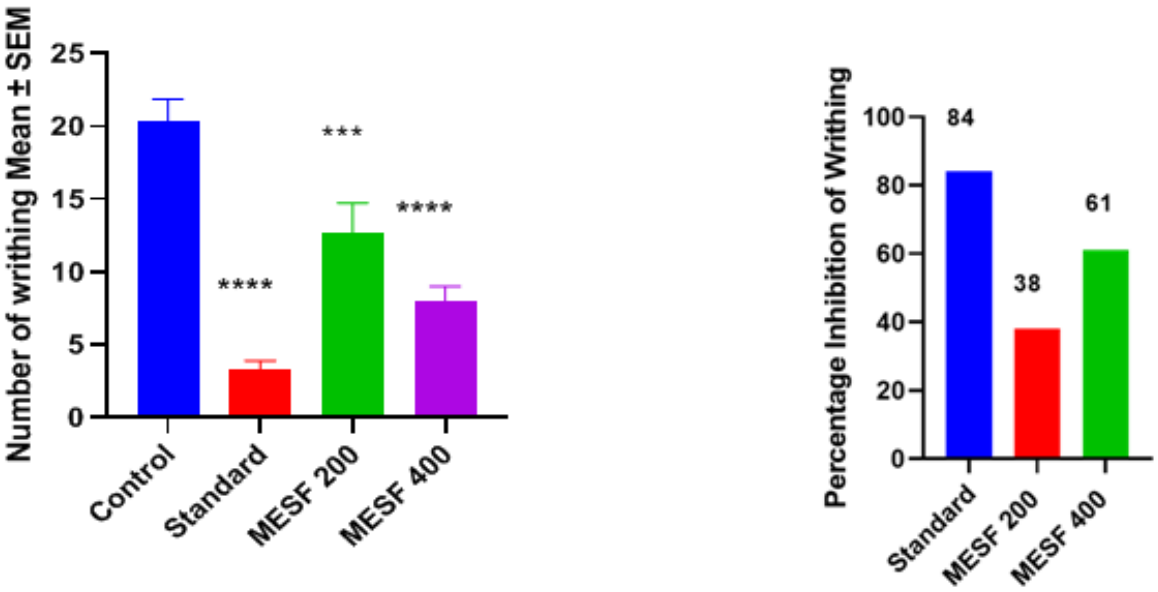


Figure 2. Effect of the oral administration of the WPCE on the ASA induced writhing test in Swiss albino mice. Here, all the values are expressed as mean ± SEM and SEM = Standard error of mean, WPCE = *W. paniculata* crude fruit extract. (*) indicates $p < 0.05$, (**) indicates $p<0.01$ and (***) indicates $p<0.001$ and ****$p < 0.0001$ compared to the normal group.

Phytochemicals like flavonoids have been additionally answered to target prostaglandin production which is involved in pyrexia[43]. In like manner, a few alkaloids and tannins are being connected with blocking pain arousement and against nociceptive movement[44-46]. The promising central and peripheral analgesic property of the plant could be the reason for its use treating pain that could have been manifested by various ailments and therefore hinting towards the innumerable application of this plant as a traditional medicinal plant etc

The antidiarrheal action of crude *W. paniculata* has been accounted for without precedent for this investigation (Figure 3. a, b).

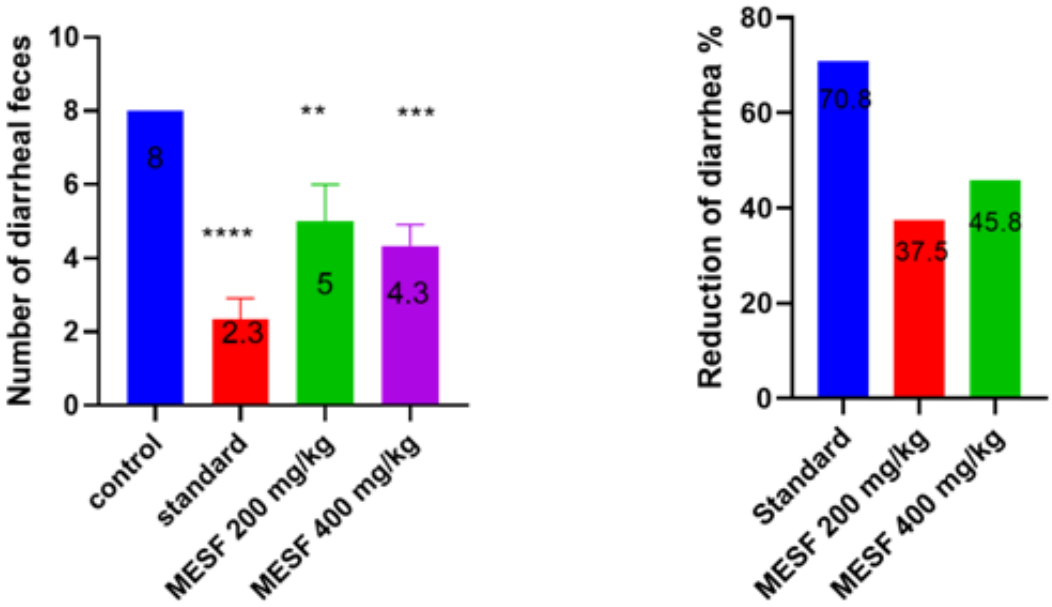


Figure 3. Effect of the oral administration of the WPCE on the castor oil induced diarrhea in Swiss albino mice. Here, all the values are expressed as mean ± SEM and SEM = Standard error of mean, WPCE = *W. paniculata* crude fruit extract. (*) indicates $p < 0.05$, (**) indicates $p<0.01$ and (***) indicates $p<0.001$ and ****$p < 0.0001$compared to the normal group.

This action means that its utilization in different stomach-related issues like cramps, constipation, indigestions and so forth. One of the different mechanisms by which castor oil initiates diarrhea is electrolyte permeability via endogenous prostaglandin secretion. One of the expected explanation by which the crude extracts shows its antidiarrheal action by restraining prostaglandin secretion in the digestive tract apparent by the way that the defecation after treatment were somewhat dry[47].

5. Conclusion

The present study revealed the hypoglycemic, analgesic and antidiarrheal effects of crude methanol of *W. Paniculata*. There is no data available till now regarding the bioactivity of this plant. Analgesic properties of this plant showed significant result which supports the use of plant during chest pain by tribal people. Moreover, the antidiarrheal activity of the plant extract is noteworthy. The hypoglycemic test showed mild activity and Consideration of bioactive fractions of the plant identified by the present study might be worthy in this manner.

6. Conflict of Interest

The authors declare that they have no conflict of interest.

7. Acknowledgement

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