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ANTIDIARRHEAL, ANTIDIABETIC, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACTS OF LEAVES OF *CLERODENDRUM VISCOSUM* (VENT.)

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ABSTRACT: The present study was aimed to investigate the possible antidiarrheal, antidiabetic, antioxidant and antimicrobial activity of methanolic extracts of leaves of *Clerodendrum viscosum* (Vent.) Different screening models are employed for an investigation like Castor oil induced diarrheal model for antidiarrheal activity screening, alloxan-induced diabetic model for antidiabetic activity screening, antioxidant activity by DPPH scavenging method and disc diffusion method for the antimicrobial activity screening. *Clerodendrum viscosum* (Vent.) shows good anti-diabetic, antidiarrheal and antioxidant activity but having no antimicrobial activity in relationship with the standard drug. *Clerodendrum viscosum* (Vent.) methanolic extract dose of 500mg/kg significantly reduces the blood glucose level at a 1st hour to 3rd hour 130 to 36 mg/dL in *in-vivo* mice model. During antidiarrheal screening, *Clerodendrum viscosum* (Vent.) extract dose reduces the number of stool from 3 to 1in 1st hour to 3rd of treatment. DPPH scavenging activity on *Clerodendrum viscosum* (Vent.) was significant with the standard. $IC_{50\%}$ 8.93 µg/mL and 7.8 µg/mL for sample *Clerodendrum viscosum* (Vent.) and standard ascorbic acid respectively.

INTRODUCTION: *Clerodendrum viscosum* (Vent.) belongs to the family Verbenaceae. Bengali/Vernacular names are Ghetu, Bhat. Tribal name**s** are (Chakma); Kho pa che, Khun kha baong (Marma); Baita gach (Garo). The plant is tonic, antipyretic and anthelmintic. Leaves and roots are used in asthma, tumors and certain skin diseases. Infusion of the leaves is used as bitter tonic and antiperiodic in malaria. The expressed juice of the leaves is laxative and cholagogue. Leaves are also used in chest complaint with a cough and difficult expectoration.

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In Rangamati, leaf-boiled water is used as a bath in jaundice by the tribal; Marmas take a bath for scabies. Root juice is warmed and rubbed on the penis to treat impotence. Root juice along with ginger is given to relieve colic pain by the Garo in Madhupur. Alcoholic extract of the young leaves possesses strong antibacterial and poor antifungal properties [1, 2].

The plant contains saponin, flavonoids, alkaloids, a new glycoside, clerodendroside, lupeol, benzoic acid derivatives, and β-sitosterol. The plant also contains clerosterol, clerodolone, clear done. Leaves contain protein, free reducing sugar, a bitter principle, clerodin a sterol, oleic, stearic and lignoceric acids, tannin, glucuronide, and gallic acid. Roots contain lupeol & ß-sitosterol, the antifungal flavonoids, cabruvin, and quercetin. The seeds contain fatty oil, in which the major fatty

acids are palmitic, oleic and linoleic acids. Clerodin and hentriacontane have been isolated from flowers [3-5].

MATERIALS AND METHODS:

Plant Material Collection and Identification: Leaves of *Clerodendrum viscosum* (Vent.) were collected from the district of Chittagong and were identified by the experts and preserved in the herbarium, department of botany, University of Chittagong.

Preparation of Extracts Preparation: The leaves were collected and clean to separate from undesirable plant parts. They were air-dried for one week. The fruits were milled into coarse powder by using laboratory milling equipment. Cold extraction was performed during extract preparation. About 250 g of powdered material was taken in a clean, flat bottomed glass container and 900 ml of 80% methanol were added in the container. The container with its contents was sealed and kept for 10 days accompanying occasional shaking and stirring to enhance the efficient extraction. The whole mixture then filtered through a piece of clean, white cotton and finally, it was filtered through Whatmann filter paper [Bibby RE200, Sterilin Ltd., UK]. The filtrate (methanol extract) obtained was evaporated under the ceiling fan and on a water- bath until dried and % yield of fruit calculated 3.57% [6]. The extract was stored at 4 ºC in the refrigerator until use.

Experimental Animals: Young Swiss-albino mice aged 4-5 weeks old and average weight 20-25 g were employed for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They were kept in a standard environmental condition (RH 55% to 60%, room temperature 25± 2 ºC and 12 h light/ dark cycle) for one week for adaptation after their purchase and fed ICDDR, B formulated rodent food and water. The experimental study was performed under the guidelines of Institutional Animal Ethics Committee [7].

Chemicals and Drugs: Drug Kanamycin, Loperamide was from square pharmaceuticals limited and analytical kits were laboratory reagent grade and from Merck Specialties Private Limited, India.

DPPH free radicals Scavenging Activity: The free radicals scavenging activity of both plants extract were measured by decreased the absorbance of the methanolic solution of DPPH (2, 2-Diphenyl-1- picrylhydrazyl). The method modified by Gupta *et al.* [8, 9] Stock solutions (1 mg/ml) of the plant extracts were prepared in methanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 µg/mL. Diluted solutions (2 mL) were added to 2 mL of a 0.004% methanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm, and from these values, the corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against concentration, and from the graph, $IC_{50\%}$ were calculated. The experiment was performed in duplicate, and average absorption was noted for each concentration. Ascorbic acid was as a positive control.

Antimicrobial Activity: The antimicrobial screening was performed using the disc-diffusion method. Sample disc 50 g/disc and Standard Kanamycin (30 µg/disc) discs were used as positive control, and blank discs were used as negative controls. The sample discs, standard antibiotic discs, and control discs were placed gently on marked zones in the agar plate's pre-inoculated with test bacteria, protozoa, and fungi. The plates were then kept in a refrigerator at 4 ºC for about 24 h to allow sufficient diffusion of materials from discs to surrounding agar medium.

The plates were then inverted and kept in an incubator at 37 ºC for 24 h. Antibacterial activity of the crude methanolic extract was determined by disc diffusion method [10-12]. The selected organisms were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Sarcinalutea, Bacillus megaterium, Salmonilaparatyphi, Sheigelladysenteriae, Bacillus subtilis, Bacillus ceracius, V. parahemolyticus, Aspergillusniger, Candida albicans, and Saccharomyces cerevaccae.*

Antidiarrheal Activity: Castor oil induced diarrheal model was followed for this experiment. The method, described by Chatterjee was followed for this study [13]. The employed mice were screened initially by giving 0.3 ml of castor oil and only those showing diarrhea was selected for the final

experiment. The test animals were selected randomly and divided into three groups and each group having three mice. They were accurately weighed & properly marked of the experimental groups.

As group-I or the control received only distilled water containing 1% Tween-80 (10 ml/kg). Group-II or the positive control received the standard anti-motility drug, Loperamide (5 mg/kg) as an oral suspension. The test group III was treated with a suspension of fruits extract of *Clerodendrum viscosum* (Vent.) at the oral dose of 100 mg/kg and 200 mg/kg body weight. Test samples, control, and Loperamide were given orally using a feeding needle.

Castor oil dose of 0.5 ml per mouse was administered to all mice, and after 1 h of administration, the mice were fed with the samples, control, and Loperamide. Individual animals of each group were placed in separate cages with adsorbent paper beneath the case and examined for the presence of diarrhea every hour in four hours of study after the castor oil administration. Stool or fluids from the mouse are the sign of induction diarrhea.

A number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour and noted for each mouse. The latent period of each mouse also counted. At the beginning of each hour, old papers were replaced for the new ones. During an observation period, the total number of stool output including diarrheic faces excreted by the animals was recorded. A numerical score based on stool consistency was assigned as follows: normal stool=1 and watery stool=2 [14-16].

Antidiabetic Activity:

Experimental Induction of Diabetes: Experimental induction of diabetes in mice, a freshly prepared solution of alloxan monohydrate in normal saline at a dose of 120 mg/kg body weight, were injected to mice intraperitoneally. Alloxan can produce fatal hypoglycemia as a result of massive pancreatic insulin release mice were treated with 20% glucose solution (5 - 10 ml) orally after 6 h. The mice were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia. After 1 week, mice with moderate diabetes that exhibited glycosuria and hyperglycemia (*i.e.*, blood glucose concentration >200 mg/dL) were taken for the experiment.

Experimental Design for Antidiabetic Activity Study: Fifteen mice were divided in to five groups as Group I: normal rats received only distilled water during the experimental period, Group II: diabetic control rats received only distilled water during the experimental period, Group III: diabetic mice administered 500 mg/kg sample, Group IV: diabetic mice administered 250 mg/kg sample, Group V: diabetic mice administered 0.25 mg/kg glibenclamide.

Treatment was continued for 6 hours following oral administration to the experimental animals by gastric intubation, using a force-feeding needle. Blood glucose was estimated on withdrawing blood samples were from tail vein before dosing (0 hours) and then a 1st h, 3rd h and 5th h respectively from all groups of mice. A fixed amount of rat chow and fluid was given to each rat and replenished the next [17, 18].

RESULT AND DISCUSSION: Antioxidant activity, % scavenging of the DPPH free radical was measured, and calculation was done, and results are summarized as **Table 1** and **Fig. 1.**

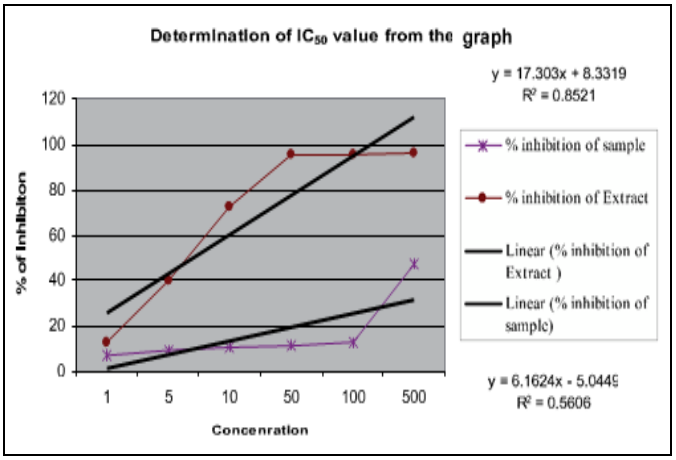

FIG. 1: GRAPHICAL COMPARISON BETWEEN SAMPLE AND STANDARD

$IC_{50\%}$ was 7.8 and 8.93 for standard and *Clerodendrum viscosum* (Vent.) respectively. *Clerodendrum viscosum* (Vent.) shows strong antioxidant property like standard. These experimental results suggest us more and more research on this plant to establish a new source of natural antioxidant.

TABLE 1: ANTIOXIDANT ACTIVITY OF *CLERODENDRUM VISCOSUM* (VENT.)

Sample	Concentration (µg/mL)	Absorbance	% Inhibition	$IC_{50\%}$
Bank (DPPH)	200	3.379	--	--
	1	2.939	13.02	
	5	2.026	40.04	
Ascorbic acid	10	0.921	72.74	
	50	0.148	95.62	7.8
	100	0.141	95.83	
	500	0.132	96.09	
	1	3.135	7.22	
	5	3.075	9.00	
Clerodendrum	10	3.026	10.45	8.93
viscosum Vent.	50	2.981	11.78	
	100	2.939	13.02	
	500	1.768	47.68	

Antimicrobial Activity: *Clerodendrum viscosum* (Vent.) has no antimicrobial activity, either bacteria or fungi. Experimental results tabulated in **Table 2**, strongly support us to say *Clerodendrum viscosum* (Vent.) leaves methanolic extract has no antimicrobial activity.

TABLE 2: ANTIMICROBIAL ACTIVITY DETERMINATION BY FOLLOWING DISC DIFFUSION METHOD

Strains	The diameter of the zone of inhibition in mm	
	***Clerodendrum viscosum* (Vent.) (50 mg/disc)**	**Kanamycin (30 µg/disc)**
Staphylococcus aureus	0	32
Pseudomonas aeruginosa	8	27
Escherichia coli	0	19
Sarcinalutea	0	31
Bacillus megaterium	7	32
Salmonilaparatyphi	0	36
Sheigelladysenteriae	0	31
Bacillus subtilis	0	28
Bacillus ceracius	0	29
V. parahemolyticus	0	32
Aspergillusniger	0	34
Candida albicans	7.1	22.2
Saccharomyces cerevaccae	0	31

0 = No growth inhibition

Antidiarrheal Activity: We tested for antidiarrheal activity of *Clerodendrum viscosum* (Vent.) leaves methanolic extract, and results show **Table 3** the antidiarrheal activity of *Clerodendrum viscosum* (Vent.). This plant leaves methanolic extract may be a good candidate of antidiarrheal agent for traditional use and drug source for diarrhea treatment.

TABLE 3: ANTIDIARRHEAL ACTIVITY OF *C. VISCOSUM* (VENT.) LEAVES EXTRACT

Group & Treatment	Number of Mice	Number of stool		
		1h	**2h**	**3h**
Normal	M 1	0	2	1
	M 2	0	1	1
	M 3	2	0	2
Control (only castor oil)	M1	6	4	2
	M2	4	3	2
	M3	5	3	2
Dose 100mg/kg	M1	2	2	1
	M2	1	1	0
	M3	2	0	1
Dose 200mg/kg	M1	3	0	1
	M2	2	1	0
	M3	2	1	0
Loperamide	M1	2	2	1
	M2	2	2	1
	M3	1	1	1

Anti-diabetic Activity: Anti-diabetic activity results are summarized as **Table 4.**

TABLE 4: ANTI-DIABETIC ACTIVITY OF *C. VISCOSUM* (VENT.) LEAVES EXTRACT

Group & treatment	Mice	Blood glucose level at different hours (after treatment)			
		0h	**1h**	**3h**	**5h**
Normal	M1	21	32	28	23
	M2	27	39	31	26
Diabetic Mice (untreated)	M1	380	364	250	311
	M2	363	337	318	308
Diabetic Mice (treated with 500mg/kg Extract)	M1	363	130	36	40
	M2	510	133	47	63
Diabetic Mice (treated with 250mg/kg Extract)	M1	556	541	470	269
	M2	546	553	188	154
Diabetic Mice (treated with 0.25mg/kg Glibenclamide	M1	559	478	253	160
	M2	541	512	280	171

CONCLUSION: In the conclusion of this study we conclude that the plant *Clerodendrum viscosum* (Vent.) leaves shows the strong antioxidant property as well as antidiabetic and antidiarrheal activity. Future research and *in-vivo* study in future may result as good source of natural antioxidant, antidiabetic and antidiarrheal drug.

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CONFLICT OF INTEREST: Nil

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