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BIOACTIVITIES OF METHANOLIC EXTRACT OF *CLERODENDRUM VISCOSUM* ROOTS NATURALLY GROWING IN BANGLADESH

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Abstract

Roots of *Clerodendrum viscosum* are profoundly used throughout the world due to its ethno-medicinal properties and folkloric use since the very beginning of medicinal treatment. Due to the potential disease preventive properties, we investigate the methanolic extracts of the roots of the plant for biological evaluation in vitro. The methanolic extract of *Cl. viscosum* roots was partitioned into ethyl acetate soluble fraction (ESF), petroleum ether soluble fraction (PSF), carbon tetrachloride soluble fraction (CTSF), chloroform soluble fraction (CSF) and aqueous soluble fraction (AQSF). The extracts were evaluated for their thrombolytic, membrane stabilizing, antimicrobial and the results were compared with standard drugs; streptokinase for thrombolytic, acetyl salicylic acid for anti-inflammatory, kanamycin for antimicrobial activities. In thrombolytic investigation, among all partitionates, the ESF showed highest % of clot lysis (54.47%) as compared to (69.13%) and (3.17%) exhibited by the standard streptokinase and water. In case of membrane stabilizing study, ESF also significantly inhibited the haemolysis of human erythrocyte membrane both induced by hypotonic solution (60.30 ± 0.54%) and by heat (44.21 ± 0.59%), respectively as compared to (69.01 ± 16%) and (71.90 ± 0.19%) demonstrated by acetyl salicylic acid. In antibacterial activity, it was exhibited by the extracts, was comparatively more prominent on the gram negative bacteria than the gram positive bacteria. Our study revealed that satisfactory amount of flavonoid and tannin presence showed a significant and positive correlation between bioactive compound contents with pharmacological activities of *Cl. Viscosum* roots.

Key words: *Clerodendrum viscosum*, thrombolytic, membrane stabilizing, antimicrobial properties.

Introduction

Medicinal plants are one of the important contributors to most of the medicinal preparations as raw plant materials, refined crude extracts, mixtures and isolated pure compound. Numerous plants species have been known to possess medicinal values and are used to treat variety of ailments in various cultures worldwide [1].Despite the great advancement observed in modern medicine in recent decades, plants still play an important role in health care [2] and according to WHO more than 3/4th of the world population relies on medicinal plants or natural products to treat common ailments. Despite the toxicity profile of most medicinal plants, which are yet to be thoroughly evaluated, it is well accepted that medicines derived from plant products are safer, low cost and easily available than their synthetic counterparts [3] Plants not only provide food, shelter and medicine but the life sustaining oxygen gas. Since disease, decay have always co-existed with life, the early man had to think about disease and its disease and its treatment at the drawn of human intellect. Thus, the human race started using plants as a means of treatment of diseases and injuries from the early days of the civilization on earth of the long journey from the ancient time of modern age the race successfully used plants and plant products as therapeutics tools for fighting against disease and various other health ailments[4]. *Clerodendrum viscosum belongs to the family* Verbenaceae it is locally known as Gatupata'. It comprises 35 genera and 1,200 species found mainly in the tropical and subtropical regions of the world [5]. The different parts of the plant *C. viscosum* have used for the restoration of different physiological hazards such as, asthma and other inflammatory diseases[6].The roots of the plant have been claimed to be used in dyspepsia, seeds in dropsy and leaves as a febrifuge and in cephalagia and ophthalmic [7]. Because of its copious, widespread availability and folkloric use, the present research was undertaken to enquire into the potentiality of *Cl.viscosum* root extract as membrane stabilizing, thrombolytic, antidiarrhoeal activity against important human pathogens.

Materials and Methods

Plant materials

The roots of *Cl. Viscosum* roots were collected from Tangail district near Dhaka, Bangladesh in February 2015 and were identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka. The Voucher specimens for this plant was preserved in Bangladesh National Herbarium office.

Preparation of extract

To avoid the loss of essential plant components, the collected plant roots were shade dried for several days and then oven dried for 12 hours at 37˚C to facilitate grinding and to obtain powder of uniform particle size that can pass through sieve # 30-40. The powdered roots were then preserved in an air- tight container. The dried powder material (390 gm) was soaked in 1400 ml of 90% methanol for twelve days and was shaken occasionally. The whole mixture was filtered by a piece of clean, white cotton followed by Whatman filter paper. The filtrate was dried using a vacuum rotary evaporator at optimum temperature of 40 ˚C to prevent loss of important plant constituents and to obtain the crude methanolic extract of *Cl. viscosum* (yield-9 gm, table 1). The concentrated aqueous methanol extract was partitioned by the Kupchan method and the resultant partitionates, i.e. ethyl acetate (ESF), petroleum ether (PSF), carbon tetrachloride (CTSF), chloroform (CSF), and aqueous soluble (ASF) extracts were used for the current investigation.

Phytochemical Screening

The freshly prepared organic extracts were qualitatively tested for the presence of various categories of phytochemicals. These were identified by characteristic color changes using standard procedures, previously described by Sofowara [8].

Determination of total flavonoid content

The total flavonoid content was estimated using a method previously described by Kumaran and Karunakaran [9] using quercetin as a reference compound. To determine total flavonoids content in this study, 1 ml of roots extract in ethanol (250 µg/ml) was mixed with 1 ml aluminum chloride in ethanol (20 mg/ml) and a drop of acetic acid. The mixture was then diluted with ethanol to 25 ml. The absorption was read after 40 min at 415 nm. A blank sample was prepared in similar way without the extract. The absorption of various concentrations of standard quercetin solution in ethanol was measured under the same conditions to plot a calibration curve.

Determination of total tannin content

The total tannin content of *Cl. viscosum* was determined by Folin - Coicalteu method [10]. Briefly, 0.3 ml (300 µl) of the methanolic extract and its different fractionates were added to a volumetric flask (10 ml) containing 2.7 ml of Folin-Coicalteu (1:10) phenol reagent. After 5 min, 2 ml of 7.5 %

sodium carbonate solution was added to each tube, the mixture were shaken, followed by heating at 45 ^{0}C for 15 minutes and was kept at room temperature for 30 min in dark place. A set of reference standard solutions of tannic acid (50 to 300 µg/ml) were prepared similarly without extract. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The total tannin content was determined from extrapolation of tannic acid calibration curve.

In vitro thrombolytic activity

Sample preparation

The crude extracts were individually suspended in 1 ml of sterile distilled water and shaken vigorously on a sonicator to obtain a uniform liquid. The suspension of the extract was kept overnight and decanted later to remove soluble supernatant, which was filtered through a filter paper to obtain residue less solution. The resulting solution used as such for *in vitro* evaluation of clot lysis activity**.**

Thrombolytic assay

The thrombolytic activities of prepared extracts were evaluated by the method described by Daginawala [11].By sterile hypodermic syringe 5 ml sterile distilled water were added in freeze- drying streptokinase (SK) vial (15, 00,000 IU) and mixed properly. This suspension used as a stock from which 100 µl (30,000 I.U) was used for *in* vitro thrombolytic activity. Venous blood (6 ml) was provided by healthy and willing volunteers, noting that they all did not have any history of oral contraceptive or anticoagulant therapy. The blood was taken maintaining all the septic conditions and precautions. In six different pre-weighed sterile vials (1 ml/vial), 1 ml of withdrawn blood was transferred and incubated at room temperature without being disturbed for 45 minutes to form clot and serum. The serum was completely removed without disturbing the formed clot and each vial having clot, were again weighed to determine the clot weight. The vials containing pre-weighed clot were separately labeled. An aliquot of 100 µl, crude extract and aqueous solutions of various fractionates were added to the different vials one by one. As a positive control, 100 µl of SK and as a negative non thrombolytic control, 100 µl of isotonic solution were separately added to the clot containing respectively. To observe clot lysis, all the vials were incubated at room temperature for 90 minutes then the released fluid was removed and vials were again weighed to observe and note down the difference in weight after clot disruption. Differences obtained in weight were taken before and after clot lysis were expressed as percentage of clot lysis as shown in the equation:

% of clot lysis = (wt. of released clot /clot wt.) × 100.

In vitro membrane stabilizing assay

The membrane stabilizing activity of the extracts were assessed by evaluating their ability to inhibit hypotonic solution and heat-induced haemolysis of human erythrocytes following the method of Omale [12].

Hypotonic solution induced hemolysis

The test sample contained stock erythrocyte (RBC) suspension (0.5 ml) with 5 ml hypotonic solution with (50 mM NaCl in 10 mM sodium phosphate buffered saline, pH 7.4) containing different fractions of methanolic extract (2 mg/ml) and acetyl salicylic acid (0.1 mg/ml). The acetyl salicylic acid was used as the reference standard. The mixtures were centrifuged for 10 min at 3000 rpm, and incubated for 10 min at a room temperature of 25 ˚C.The absorbance of supernatant content haemoglobin was measured at 540 nm using UV spectrophotometer. The percentage inhibition of either haemolysis or membrane stabilization was determined using the following equation:

$$\% \text{ inhibition of haemolysis} = 100 \times \{(OD_1 - OD_2)/OD_1\}$$

Where, OD_1= Optical Density of hypotonic buffered saline solution alone (control) and OD_2= Optical density of the test sample in hypotonic solution.

Heat induced haemolysis

Isotonic buffer solution containing 2 mg/ml of different partitions of *Cl. viscosum were* placed into two centrifuging tubes [13]. These two sets of control tubes contained 5 ml of sterile vehicle and 5 ml of acetyl salicylic acid (0.1 mg/ml) respectively Erythrocyte suspension (30 ul) was added to each tube separately and mixed gently by inversion. One pair of tubes was incubated at 54 ˚C for 20 min in a water bath. The other pair was maintained at 0-5 ˚C in an ice bath. The reaction mixture was centrifuged for 10 min at 3000 rpm and the absorbance of the haemoglobin content in supernatant was measured at 540 nm, and the results obtained were recorded.

Statistical analysis

Three replicates of each sample were used for each test to facilitate statistical analysis and the data were

presented as mean ± standard deviation (SD).

Antimicrobial activity

Test bacteria

The samples were examined for antimicrobial activity by the standardized disc diffusion method. A total of nine bacterial strains were used in the present study. Five Gram negative strains namely *Escherichia coli, Proteus mirabilis, Pseudomonas fluroescens*, *Pseudomonas aeruginosa, Salmonella typhi.* Four Gram positive bacteria species viz. *Staphylococcus aureus*, *Bacillus subtilis, Sarcina lutea* and *Bacillus cereus* were used to investigate the antibacterial potential of the roots' extracts. These pathogenic strains were obtained from the Department of Microbiology, University of Dhaka. The bacterial strains were maintained on nutrient agar slants tubes at 4 °C at all times.

Determination of MICs

The minimum inhibitory concentrations (MICs) of the roots extract was carried out by using seven test organisms; *Escherichia coli, Shigella dysentria, Staphylococcus aureus*, *Bacillus subtilis, Salmonella typhi. Bacillus cereus* and *Sarcina lutea* using the procedure with slight modifications [14]. An aliquot of 500 µl of extracts were diluted serially to 0.5-2000 µg/ml, a sample of test organism previously standardized to 0.5 as McFarland turbidity standard (1.5×10^8 CFU/ml; are used as a reference to adjust the turbidity of bacterial suspensions) was introduced into the tubes containing 2 ml of sterile nutrient broth followed by tubes were then incubated at 37 °C for 24 h. Bacterial growth was examined and recorded by observing the rate of turbidity. The lowest concentration of extract (maximum dilution) exhibited no bacterial growth. The procedure was repeated in triplicates and compared with the extract free standard antibiotics kanamycin.

Antibacterial activity

The prepared extracts were screened for their antibacterial activity in comparison with standard kanamycin (30 µg/disc) *in vitro* by disc diffusion technique using various bacterial strains [15]. Nutrient agar was sterilized in a flask to avoid sepsis and cooled to 45-50 °C and then taken in sterilized, autoclaved petridishes with a diameter of 120 mm. The paper discs (6 mm diameter, Whatman No. 1 filter paper) containing 100 µg/ml plant extract. Each extract was dried on to separate paper disc and placed aseptically on the agar surface with the help of a sterile forceps and paper discs were pressed slightly with the forceps to make complete contact with the surface which is previously inoculated agar plates with the test microorganisms [16]. The petridishes were kept at 4 °C for 12 h. followed by the incubation at 37 °C for 16 h for the growth of the microorganisms. The inhibition zone around each disc was measured in nearest millimeter and the assay was performed in triplicate for each extract for accuracy. The results were recorded by measuring the zone of growth inhibition of microbes surrounding the disc.

Results and discussion

The results of qualitative phytochemical screening of various extracts of *Cl. viscosum* roots are presented in (table 2) which showed presence of important classes of plant secondary metabolites. All the prepared extracts showed the presence of alkaloids, carbohydrates, tannins and flavonoids while steroids were detected only in Petroleum soluble fractions.

Total flavonoid and tannin content

Since, all the partitionates demonstrated positive results for flavonoid and tannin in the preliminary phytochemical screening; the same is quantified by the analysis of total tannin and flavonoid content. Aluminum chloride colorimetric method was used to determine the total flavonoid content in the plant extract of different fractionates of the *Cl. viscosum.* This method is based on the quantitative determination of the flavonoid-aluminum complex between flavonoid of the crude extract and aluminum chloride [17].The total flavonoids contents of roots of *Cl. viscosum* were calculated using the linear equation obtained from the standard curve of quercetin ($y = 0.0098x - 0.0364$; $R^2 = 0.9724$) and expressed as quercetin equivalents (QAE) per gram of the plant extract. In this investigations, ESF revealed the highest flavonoid content (50.37 ± 0.25 mg/gm) while PSF was found with the lowest flavonoid content (39.44 ± 0.37 mg/g). The tannin content was examined in extracts using the Folin-Coicalteu reagent and is expressed in terms of tannic acid as mg of TAE/gm equivalent (the standard equation $Y=0.0999x-0.0161$.$R^2=0.9996$). The total tannin content was also the most significant in ethyl acetate soluble fraction (40.18 ± 0.22 mg /gm) whereas methanolic extract was (21.98 ± 0.59mg/gm) lowest in amount (table 3).

Thrombolytic activity

The results of *in vitro* thrombolytic activity revealed that addition of a positive control (a fibrinolytic drug) to the clots showed 69.23% lysis of clot on the other

hand sterile distilled water a, negative control exhibited a negligible percentage of lysis of clot 3.07%. The % clot lysis by various extracts were observed in the following order, ESF (54.47%) <PSF (51.30%) <CSF (45.5%) <ME (30.18) <AQSF (18.80%). In our thrombolytic assay, negative control clearly demonstrated that negligible percentage of lysis of clot (3.17%) when water was added to the clot whereas a positive control (a fibrinolytic drug) showed 69.13% lysis of clot. So it is clear that significant (p value < 0.001) percentage of thrombolytic activity was exhibited by all extract of Cl. viscosum, are presented in the (Table 4). The possible mechanism of clot lysis is; briefly**,** Streptokinase (SK) is an enzyme secreted by several species of streptococci that can bind and activate human plasminogen. Standard SK is used as an effective and inexpensive thrombolysis medication in some cases of myocardial infarction (heart attack) [18] and pulmonary embolism [19]. It is a medications well known as fibrinolytic and bind with human plasminogen. Complexes of streptokinase with human plasminogen can hydrolytically activate other unbound plasminogen by activating through bond cleavage to produce plasmin [20]. Plasmin is an important enzyme (EC 3.4.21.7) present in blood that degrades many blood plasma proteins, including fibrin clots. The degradation of fibrin is termed fibrinolysis. It is evidenced that plants which have plasminogen receptors that bind plasminogen and easily activated to plasmin, which could lead to fibrinolysis [21]. The results from the study clear that *Cl. viscosum* extract potentially lysis of clot as positive control.

Membrane stabilizing activity

The *in vitro* membrane stabilizing activities of different partitionates of crude extracts of *Cl. viscosum* significantly protected the haemolysis of HRBC membrane induced by hypnotic solution and heat as compared to the standard ASA. The membrane stabilizing activity against hypnotic solution was observed in the following order ASA (71.90 ± 0.19%), ESF (60.30% ± 0.64), PSF (45.30 ± 0.31%), CSF (40.45 ± 0.77) and AQSF (21.30% ± 0.51), respectively. In heat induced method, the values of ASA (70.12 ± 0.26), CSF (40.23 ± 0.57 %) and ESF (56.21 ± 0.69%) were decreased slightly but for, AQSF (22.34% ± 0.42) and PSF (47.30 ± 0.61) a little increase in membrane stabilizing activities was noted (table 4). The exact possible mechanism for the membrane stabilizing effect of *Cl. viscosum* roots were not known. However, a number of studies have shown that flavonoids and other phenolic compounds which exhibited analgesic and anti-inflammatory effects [22]. Due to presence bioactive compounds in extract has various effects on mammalian cellular system and structures and has shown to protect biological membrane against free radical-induced oxidative damage and inflammation [23] Presence of significant amount of phenolic compound in the extract of *Cl. viscosum* indicated that *Cl. viscosum* has potential to inhibit of hemolysis induced by hypotonic solution as well as by heat, which leads to protection of haemolysis of HRBC membrane [24]. From the literature reviewed also found that, bioactive constituents as flavonoids, exhibited anti-inflammatory effects as a result of their membrane stabilizing action in various experimental animal models [25].

Assessment of minimum inhibitory concentrations (MICs)

The results of MICs determination of the crude extract of *Cl. viscosum* are shown in the (table 6). The MICs value of extracts varied between 11-55 µg/ml. The highest MIC values of the crude extract was 55 µg/ml against *Bacillus cereus* followed by *Escherichia coli* (14 ± 0.22); *Staphylococcus aureus (*11 ± 0.11), *Shigella dysentria* (25 ± 0.01), *Salmonella typhi* (35 ± 0.29), *Sarcina lutea* (38 ± 0.81)*, Bacillus sublitis (27* ± 0.61) whereas the lowest MIC values was (11 ± 0.81) against *Staphylococcus aureus.*

Antimicrobial activity

The antibacterial activity of the methanolic extract of roots of *Cl. Viscosum* root was studied against both gram positive and gram negative species at concentrations (100 µg/ml) and the antibacterial activity was compared with the standard kanamycin (35 µg/mL). Results were recorded as presence or absence of zones of inhibition around the disc. The inhibitory zone around the disc indicated the absence of test bacterial growth and is reported as positive and the absence of zone as negative. The results of antibacterial screening of petroleum ether, chloroform, carbon tetra chloride, methanol extract, and water extracts of *Cl. viscosum* are presented in (table 5). The results revealed variability in zone of inhibition of each extract against a given bacteria. Among the various extracts used, petroleum ether extracts of *Cl. viscosum* showed the highest activity (zone of inhibition; 18 ± 0.31 mm) against *Bacillus cereus* in comparison to the standard (29 ± 0.14).The lowest activity (zone of inhibition; 9 ± 0.41 mm) of CSF was observed against gram positive species *Staphylococcus aureus.* PSF extract appeared to be

most effective extract against *Bacillus cereus*. CTSF extract exhibited good to moderate antibacterial activity against almost all bacterial strains (8 ± 0.14 - 12 ± 0.67 mm) except *Shigella dysenteriae* at the concentration of 100 µg/ml. The most prominent effects of CTSF were observed on gram positive bacteria, *Bacillus cereus* and *Sarcina lutea.* Antibacterial effects of CSF were more pronounced against gram negative bacteria in comparison to gram positive. CSF was found to be inhibit growth of all gram negative microorganisms (9 ± 0.11- 13 ± 0.11 mm) while no inhibitory effects were observed against gram positive bacterial namely *B. subtilis* and *S. lutea*. No antibacterial activity was shown by water extract. The antibacterial activity was comparatively more prominent on the gram negative bacteria than the gram positive bacteria though gram positive species *Bacillus cereus* was the only strain which was sensitive to all extracts. We can claim that the roots of *Cl. viscosum* could be used against both gram positive and gram negative pathogens. From the previous research and literature reviewed found, that medicinal plants have been shown to have as bactericidal, synergistic activity and the ability to suppress bacterial virulence[26].The inhibitory effect of the extract on the growth of microorganisms could be attributed to the presence of phytochemicals that were present in the plant extract of *Cl. viscosum* . The demonstration of antibacterial activity against both positive and gram negative bacteria by this plant may be indicative of the presence of broad or narrow spectrum antibiotic compounds by affecting bacterial protein synthesis.

Correlation of total phenolic compound content with pharmacological activities of Cl. viscosum

For bioactive compound content a significant but marginal positive correlation (value of, **P < 0.001;*P<0.005; a P<0.05 was statistically significant as compared with thrombolytic, membrane stabilizing and antimicrobial activities of methanolic extract) was found. This showed a good relationship between bioactive compound content with the thrombolytic, membrane stabilizing and antimicrobial activities of *Cl. Viscosum* roots.

Conclusion

Qualitative phytochemical screening of various extracts of *Cl. viscosum* roots showed presence of satisfactory amount of bioactive compounds. Thorough analyzed the results, revealed that the plant possess thrombolytic, membrane stabilizing and antimicrobial properties, are mainly due to bioactive compound. It may be assumed and stated that extracts can be considered as good source of antimicrobial, thrombolytic membrane stabilizing remedy.

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Competing interests

The authors declare that they have no competing interests.

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Table 1. Fractions of 9 gm methanolic crude extract of *Cl. viscosum*

Plant part	**Roots**				
Extract fraction	**PSF**	**CTSF**	**CSF**	**ESF**	**ASF**
	2.3	0.7	1.6	2	2.4
Fraction amount (%)	25.5	7.7	17.7	2.2	26.6

Table 2. Results of phytochemical constituents of methanolic extract and different Partitionates of *Cl. viscosum* roots.

Test for	**PSF**	**CSF**	**ESF**	**CTSF**	**EE**
Carbohydrates	-	+	+	+	+
Reducing sugar	-	+	+	-	-
Steroid	+	-	-	-	-
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+

+ indicate present, -indicate absent

Table 3. Total flavonoid and tannin content of methanolic extract and various extracts of *Cl. viscosum*

Extracts content	**Total flavonoid content (mg QAE/gm) of dry extract**	**Total tannin content (mg of TAE/gm of dry extract**
ESF	50.37 ± 0.25	40.18 ± 0.22
CSF	49.76 ± 1.80	28.09 ± 0.11
PSF	39.44 ± 0.47	38.47 ± 0.03
ME	-	21.98 ± 0.59

Results are expressed as mean ± SD (n = 3)

Table 4. Percentage (%) inhibition of heat and hypotonic solution induced haemolysis of erythrocyte membrane and thrombolytic activity (in terms of % of clot lysis) of different fractions of *Cl. viscosum*

Samples	**% Inhibition of haemolysis**		**% of clot lysis**
	Heat induced	**Hypotonic solution induced**	
ESF	56.21 ± 0.69*	60.30 ± 0.64**	54.47**
CSF	40.23 ± 0.57	40.45 ± 0.77*	45.5**
PSF	47.30 ± 0.61	45.30 ± 0.64*	51.30**
AQSF	22.34 ± 0.42	21.30 ± 0.51*	18.80*
ASA	69.16 ± 0.26*	71.90 ± 0.19*	-
SK	-	-	69.23**
ME	-	-	30.18*
WATER	-	-	3.07*

Values are expressed as mean ± SD (standard deviation);*P < 0.005, **P < 0.001; statistically significant as compared to positive control and negative control.

Table 5. Antimicrobial activities of *Cl. viscosum root* extract against gram positive and gram negative bacteria.

Test organism	Conc. µg/ml	Zone of inhibition (in mm) ± SD (n = 3)				
		CTSF	PSF	CSF	EE	Kanamycin 30 µg/disc
Gram-positive bacteria						
Bacillus cereus	100	12 ± 0.12	18 ± 0.31	11 ± 0.11	7 ± 0.11	29 ± 0.11
Bacillus subtilis	100	11 ± 0.61	11 ± 0.91	-	10 ± 0.21	27 ± 0.91
Staphylococcus aureus	100	9 ± 0.21	-	9 ± 0.41	-	30 ± 0.31
Sarcina lutea	100	12 ± 0.81	-	-	12 ±.0.11	25 ± 0.61
Gram-negative bacteria						
Salmonella typhi	100	8 ± 0.14	13 ± 0.11	9 ± 0.11	-	36 ± 0.31
Vibreo parahemolyticus	100	10 ± 0.9	11 ± 0.65	10 ± 0.18	-	37 ± 0.11
Escherichia coli	100	12 ± 0.91	-	12 ± 0.88	12 ± 0.33	23 ± 0.18
Vibreo minicus	100	12 ± 0.67	-	10 ± 0.88	10 ± 0.11	28 ± 0.12
Shigella dysentria	100	-	10 ± 0.29	13 ± 0.11	11 ± 0.23	29 ± 0.19

Table 6. Minimum inhibitory concentrations of the crude extract of *Cl. viscosum*

Name of bacteria	MIC (µg/ml)
Escherichia coli	14 ± 0.22
Staphylococcus aureus	11 ± 0.81
Bacillus sublitis	27 ± 0.81
Shigella dysentria	25 ± 0.01
Salmonella typhi	35 ± 0.29
Bacillus cereus	55 ± 0.11
Sarcina lutea	38 ± 0.61

Standard deviation (n=3)