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Mechanical prevention of virulent *Vibrio cholerae* bacteria in some commercially dried fishes through cumin seed (*Nigella sativa)* extracts

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Abstract
The present study was designed to investigate the antibacterial activity of black cumin seed extracts with the aim at inhibiting of the growth of pathogenic bacteria, *Vibrio cholerae* on three different dried fishes named Loitta (*Harpodon nehereus),*Shrimp *(Penaeus monodon) and* Churi *(Trichiurus haumela*). Two different extracts with ethanol and acetone solvents were prepared separately. The antibacterial activities of these extracts were determined by three different methods; i. enumerating the bacterial load before and after extracts treatment, ii. Disc diffusion method, iii. Tube dilution method. A total of ten samples were taken for each method with three replicates. The selective Thiosulfate-citrate-bile salts-sucrose agar media (T.C.B.S Cholera) was used for isolation of *V. cholerae* from the dried fish tissue. The average loads of the *V. cholerae* in Loitta and Shrimp were 3.1×10^4 CFU/g and 2.67×10^3 CFU/g respectively while no *V. cholerae* was detected in dried Churi fish. Though both extracts showed strong sensitivity to the isolated bacteria, but acetone extract of black cumin was found to be more effective than ethanol extract. The Minimum Inhibitory Concentrations (MIC) value of acetone and ethanol extracts in inhibiting the growth of *V. cholerae* was 25% and 30% respectively. This result suggests that, black cumin seed extract can be used as an alternative medicine to prevent the enteric disease of consumers associated with dried fish consumption.

Keywords: black cumin, pathogenic, bacteria, antibacterial etc.

1. Introduction
Fish and seafood constitute an important food component for a large section of the World population. It has become an increasingly important source of protein and other element necessary for the maintenance of and healthy body. They come after meat and poultry as staple animal protein foods where fish forms a cheap source of protein [1]. Drying is the simplest and ancient method to preserve fish. It is a traditional method of seafood preservation employed in many countries and considered as the least expensive method of fish preservation. Dried fish is very popular and have long been consumed as a traditional food. Dried fish is a rich source of proteins; containing 80-85% protein [2-3]. Mainly due to unhygienic processing, use of spoiled fish for processing, inadequate salting, unhygienic drying and lack of air tight packing of the dried fish's quality deteriorated massively [4]. Quality control procedure is not maintained for local market [5]. So it is very important to analysis microbiological quality of dried fishes in retail trade for guarding health and hygiene of local consumer.
Among all the bacteria *Vibrio cholerae* is one of the pathogenic bacteria that may result food intoxication by consuming the affected food. Although cholera is primarily known as a water-borne intestinal disease in the endemic regions including Bangladesh. *V. cholerae* can be classified into two strains: *V. cholerae* O1 (cause cholera), whereas strains in other group, non-O1 *V. cholerae*, are generally associated with milder illness. The virulence of *V. cholerae* O1 is determined primarily by the presence of a protein enterotoxin, cholera toxin (CT). Strains that do not produce cholera toxin (i.e., are not toxigenic) tend to be a virulent or have reduced virulence but cause food poisoning with symptoms of diarrhoea, stomach cramps and vomiting [6-7]. *Nigella sativa* produces black cumin seeds, popularly known in Bengali as Kalogira, is an essential ingredient in the Asian cuisine. For thousands of years, the seeds of *N. sativa* (family: Ranunculaceae) has been used as a medicine and health-promoter.

Black cumin seeds were found to exhibit antibacterial activity against wide range of gram-positive and gram-negative bacteria and also antiviral activity [8]. It is recommended for a wide range of ailments, including fever, cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, dysmenorrhea, obesity, diabetes, paralysis, hemiplagia, back pain, infection, inflammation, rheumatism, hypertension, and gastrointestinal problems such as dyspepsia, flatulence, dysentery, and diarrhea [9-10]. It has also been used as a stimulant, diuretic, lactagogue, anthelmintic, emmenagogue and carminative [10].

Black cumin seeds and its oil have been widely used for reducing blood pressure, cleansing and notifying the liver, reducing fluid retention, supporting healthy digestion, stimulating the appetite and treating skin disorders. Also used to regulate the immune system, kill microorganisms, reduce inflammation, inhibit spasmodic activity, and open the tiny air passages in the lungs. Cumin seeds are also known to act synergistically with antibiotics [11].

A few studies on antioxidant activity of black cumin seeds [12-13], and shoots and roots have been accounted recently but there are no pertinent studies on antibacterial activity of black cumin seeds [14]. Consequently, the current study was to assess the antibacterial effects of two polar solvent extracts (acetone and ethanol) of black cumin seeds against on mechanical prevention of *Vibrio cholerae* in three different commercially important dried fish by using different in vitro methods. In this study, the antibacterial effects of two polar solvent extracts (acetone and ethanol) of black cumin seeds were assayed against *V. cholerae* the isolated from dried fish under laboratory conditions.

2. Materials and Methods

2.1 Sample collection

Three types of commercially valuable dried fish were selected. Dried fish LOITTA (*Harpodon nehereus*), CHINGRI (*Penaeus monodon*) and CHURI (*Trichiurus haumela*) were collected in sterile plastic bags separately and the bags were teighed after collection to prevent extraneous contamination. Then the collected samples were carried out to the laboratory and preserved at 4°C.

2.2 Black cumin seeds collection

About 500 g black cumin seeds (*Nygella sativa*) were bought from the spice market and sorted for separation of dirt and unwanted materials. The seeds were washed thoroughly with clean water and air dried at room temperature. Then the seeds were brought to the laboratory and preserved at room temperature in sterile plastic bag.

2.3 Preparation of extracts from black cumin seed *(N. sativa)*

The black cumin seed was heated in oven at 50º C for 15 minutes and grinded by using mixer grinder (Capacitor start motor, WUHU motor factory, China). Approximately 300 gm of powdered cumin seed was taken into two sterile conical flasks containing 150 gm each. Then 350 ml of 100% ethanol and acetone were added into flasks separately at a ratio of 1:3. The mixtures were kept at room temperature for 72 hours. The mixtures were stirred every 24 hour using sterile glass rod. Then the mixtures were filtered through Whatman® No.1 filter paper. Filtration procedures were done further twice for complete extraction of the bioactive compounds. The filtrates were then collected in separate beaker and concentrated by evaporating the solvents. Approximately after 96 hours stock solution of the extract was ready to experiment. The liquid extracts were kept in a refrigerator (-20ºC) for further study. The process was followed by Yasni., *et al*, 2009 [15] with slight modification.

2.4 Preparation of extract at different concentrations

10%, 15% and 25% concentration of extract in both solvent (ethanol and acetone) was made separately by adding 100µl, 150µl and 250µl both extracts into 900µl, 850µl and 750µl of their relevant solvents (acetone and ethanol) respectively. After adding to the solvent, mixing was done in unidirectional manner by a vortex mixer.

A serial two fold dilution of ethanol and acetone extracts ware done to get concentrations of 50%, 25%, 12.5%, 6.25% and 3.12% for MIC (minimum inhibitory concentration).

2.5 Isolation and Identification of *Vibrio cholerae*

To isolate and enumerate specific pathogen of *V. cholerae* dried fishes were cut into small pieces with a sterile scissor and weighted in electric balance (HR-200). About 2 grams of samples were collected from each dried fish. The sample was then minced and grinded properly with alkaline peptone water using mortar and pestle. The mixture was taken into eppendorf tubes with alkaline peptone water used for isolating *V. Cholerae*. Two successive selective enrichments were done using alkaline saline peptone water (ASPW) for 6hr at 37˚C followed by 18hr at 41˚C. The plates were examined for the presence of typical colonies of presumptive *Vibrio* sp. [16]. The enumeration was done in Thiosulfate Citrate Bile and Sucrose sugar (TCBS) agar medium after incubation of 24-48hr at 37˚C.). The standard plate was selected and counted the colonies. To identify *V. cholera* various biochemical test viz gram staining, motility, triple Sugar Iron (TSI) agar test, indole test, methyl red (MR) test, oxidase test. salt tolerance test with varying amounts of NaCl (0%, 1%, 3%, 6% and 8% were conducted [17-18]. All cultures of bacteria subsequently grown from stored stocks were streaked to get single colony prior to use. The bacteria were cultured on TCBS plates, nutrient agar plate and incubated at 37°C.

2.6 Experimental Design for *In-vitro* Challenge Test of black cumin against *Vibrio choleae.*

At first the bacteria enrichment stock solution of each sample was done [16]. Then 0.15 ml of 50%, 40%, 35%, 30%, 25% and 15% extracts (acetone and ethanol) of black cumin was separately mixed with 0.85 ml of test solution. 0.1ml suitable dilution of the mixture was inoculated in petri dishes that containing TCBS Cholera agar media after at subsequent interval of 2 hour up to 6 hour. This procedure was done 2 times. Test solution of sample without extract also inoculated TCBS cholera agar media at 0 hour, 2 h, 4 h and 6 h subsequently. All the inoculated TCBS agar plates were incubated at 37° C for 24 ± 3 hours. Standard plates count was done after incubation and compare the plate with and without extract.

2.7 *In-vitro* Challenge Test and Determination of Antagonistic Activity of black cumin extracts against *V. cholera*

2.7.1 Process A: Enumerate the Load before and after Treatment

At first one loop of single colony of *V. cholerae* was dissolved in separately 1ml alkaline peptone water (APW) in

eppendorf tube. Then serial dilution (ten folds) was done. After that 0.15 ml of 50%, 40%, 35%, 30%, 25%, 20%, 15% of both ethanolic and acetonic extracts of black cumin seed were mixed with 0.85ml of bacterial solution separately. Then 0.1ml of the mixture was inoculated in petri-dishes that containing TCBS Cholera agar media. Test bacterial solution without extract also inoculated in the TCBS Cholera agar media. All inoculated TCBS agar plates were incubated at 37°C for 24±3 hours. Standard plates count was done after incubation and compare the plates with and without extracts.

2.7.2 Process B: Determining the Zone of Inhibition of black cumin extracts against *V. cholerae*

Antibacterial activity of the black cumin extract was evaluated using disc diffusion method [19] with slight modification. The discs 4mm of Whatman® paper were prepared for negative control. Standard antibiotic discs were used as a positive control to compare the antibacterial activity of black cumin. TCBS Cholera agar media was prepared and raised temperature up to 100°C in hot plate. Then the media transferred into the petri dishes and kept for cooling. 0.1ml of bacterial stock solutions were placed on separate petri dishes and spread throughout the plate by spread plate technique. The antibiotic and the discs that's were loaded with 20µl of different concentration (50%, 40%, 30%, 20%) of both ethanolic and acetonic extracts separately were placed on the bacterial solution inoculated plate with help of sterile forceps carefully with adequate spacing between each other. The plates were kept at room temperature for 30min, which helps to diffuse the extract on the medium. Later the plates were then incubated at 37°C for 24hrs in incubator to determine the antibacterial activity of ethanolic and acetonic extracts of black cumin. Karamycin was used for Positive control and sterile Whatman® paper was used for negative control. After incubation, zone of inhibition in diameter was measured by a slide calipers (Tricle Brand) and recorded.

2.7.3 Process C: Determining the Minimum Inhibitory Concentration (MIC) of black cumin extracts

MIC was determined in broth serial dilution method. It is a technique in which test tubes holding identical volumes of broth with antimicrobial solution in incrementally increasing concentration are inoculated with known number of bacteria (EUCAST Discussion.5.1, 2003). Nutrient agar and McFarland standard was prepared by standard method [20]. The procedure described by Rollins, *et al*., 2003 [21] was followed with slight modification.

3. Results and Discussion

3.1 *V. cholerae* load in fish tissue of the collected dried fish

A selective media Thiosulfate-citrate-bile salts-sucrose agar (T.C.B.S Cholera medium) was used for isolation of *V. cholerae.* After sample preparation, fish tissues were taken into eppendorf tubes and APW (alkaline peptone water) was used for isolating *V. cholerae.* After the incubation, colonies for *V. cholerae* on the agar media appeared as yellow, shiny colonies, 2 to 4 mm in diameter. The average load of *V. cholerae* in the dried fish tissue of LOYTTA (*H. nehereus*) was 3.1×10^4 and in dried CHINGRI (*P. monodon*) was 2.67×10^3. No *V. Cholerae* was found in dried CHURI (*T. haumela*). The *V. cholerae* load in three dried fishes is shown in Table 01.

Table 1: *Vibrio cholerae* load in dried fish

Dried fish	Bacterial Load (CFU/g)	Avg. *V. cholerae* load (CFU/g)
Loitta (*H. nehereus*)	1.29×10^4	3.1×10^4
	7.1×10^4	
	9.2×10^3	
Chingri (*P. monodon*)	3.17×10^3	2.67×10^3
	1.83×10^3	
	3.00×10^3	
Churi (*T. haumela*)	NF	NF

N.B. NF= Not found

3.2 *In-vitro* Challenge test of black cumin against *V. cholerae*.

The present study showed that there was a significant antagonistic effect of black cumin seed (*Nygella sativa*) against *Vibrio cholerae*. *In-vitro* challenge test of dried Loitta (*H. nehereus*), the potential antagonistic effect of black cumin extract (both acetone and ethanol) was gradually obtained at 0 h, 2 h, 4 h and 6 h after treatment.

3.2.1 *In-vitro* Challenge test of black cumin acetone extract against *V. cholerae*

Average load of *V. cholerae* without extract were 7.1×10^4, 6.2×10^4, 8×10^4 and 1.64×10^4 CFU/g at 0 h, 2h, 4h and 6h respectively. With 5%, 10% and 25% concentration of extract the average load were 3.5×10^4, 8×10^3 and 2.5×10^3 CFU/g at 0 h. Average *V. cholerae* load without extract gradually increased with time but the average load of the bacteria with different concentration of extract gradually decreased.

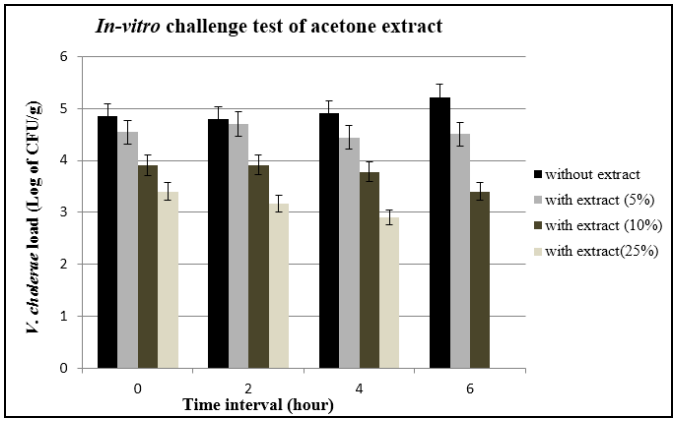

Fig 1: *In-vitro* challenge test of black cumin extract in acetone against *V. cholera*

With 5%, 10% and 25% concentration of extract the average load were 5×10^4, 8.2×10^3 and 1.5×10^3CFU/g at 2 hours and 2.8×10^4, $6\times10^3$and 8×10^2 CFU/g at 4 hours respectively. With the treatment of 25% or 250µl/ml acetonic extract of black cumin, the growth totally stopped at 6 h. From the results it could be concluded that more concentration of extract was more effective against *V. cholerae*. 25% black cumin extract was more effective to use concentration basis.

3.2.2 *In-vitro* Challenge test of black cumin ethanolic extract against *V. cholerae*

Average load of *V. cholerae* without extract were 7.1×10^4, 6.2×10^4, 8×10^4 and 1.64×10^4 CFU/g at 0 h, 2h, 4h and 6h respectively. With 5%, 10% and 25% concentration of extract the average load were 3.3×10^4, 2.6×10^4 and 1.93×10^3 CFU/g

at 0 h. Average *V. cholerae* load without extract gradually increased with time but the average load of the bacteria with different concentration of extract gradually decreased.

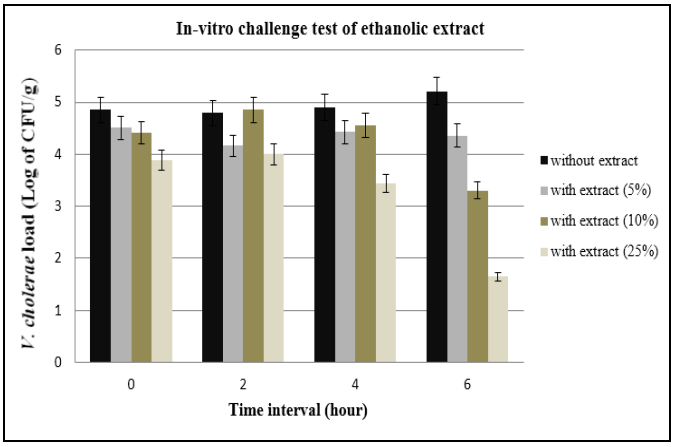

Fig 2*: In-vitro* challenge test of black cumin extract in ethanol against *V. cholera*

With 5%, 10% and 25% concentration of extract the average load were 1.46×10^4, 7.2×10^4 and 1.2×10^4 CFU/g at 2 hours and 2.7×10^4, $3.6\times10^4$and 2.5×10^3 CFU/g at 4 hours respectively. With the treatment of 25% or 250µl/ml ethanolic extract of black cumin, the growth drastically reduced at 6 h to 4.5×10^1CFU/g.

3.3 *In-vitro* Challenge test and Determination of Antagonistic Activity of Black cumin extracts against *V. cholerae.*

Process A. Enumerate the load before and after treatment

3.3.1 The antibacterial activity of Black cumin extracts in acetone against *V. cholerae*

The antibacterial activity of black cumin extracts in acetone is shown in Table 3 by comparing the average load of the bacteria without and with black cumin acetonic extract at 50%, 40%, 30% and 20% concentration. No growth of *V. cholerae* was found up to 30% concentration of extract (Table 2). In 20% concentration the load found was 2.41×10^4 that decreased in 94.6% comparing the primary load.

Table 2: *V. cholerae* load before and after treatment with black cumin acetone extract

SL. No.	Without extract		With black cumin acetone extract at different concentration							
			50%		40%		30%		20%	
	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)
01.	3.96×10^5	4.5×10^5	N	N	N	N	N	N	2.7×10^4	2.41×10^4
02.	2.68×10^5		N		N		N		1.92×10^4	
03.	6.86×10^5		N		N		N		2.58×10^4	

N.B. Avg. = average; SL. No. = Serial number; N = No growth, CFU = Colony Forming Unit. Each time 100µl solution was inoculated in Petri dish.

The procedure was repeated twice and additionally, three more concentrations of the extract (35%, 25% and15%) were prepared to determine the antibacterial activity. The average load found in 25% of acetonic extract was 5.6×10^2 (Table 03) which indicated the reduction of *V. cholerae* load in 99.8%. But at the 15% concentration average load found was 3.21×10^5 that indicated a mere reduction of load in 28.6%.

Table 3: *V. cholerae* load before and after treatment with black cumin acetone extract at 25% and 15% concentration.

SL. no	Without extract		With black cumin acetone extract at different concentration			
			25%		15%	
	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)
01.	3.96×10^5	4.5×10^5	8×10^2	5.6×10^2	3.56×10^5	3.21×10^5
02.	2.68×10^5		4×10^2		2.07×10^5	
03.	6.86×10^5		5×10^2		4.08×10^5	

N.B. Avg. = average; SL. No. = Serial number; CFU = Colony Forming Unit. Each time 100 µl solution was inoculated in Petri dish.

3.3.2 The antibacterial activity of Black cumin ethanolic extracts against *V. cholerae*

The antibacterial activity of black cumin extracts in ethanol is shown in Table 04 by comparing the average load of the bacteria without and with black cumin ethanolic extract at 50%, 40%, 30% and 20% concentration. No growth of *V. cholerae* was found up to 40% concentration of extract. The average load of the bacteria reduced at 99.7% and 12.8% in 30% and 20% concentration of the extract respectively.

Table 4: *V. cholerae* load before and after treatment with black cumin ethanol extract

SL. no	Without extract		With black cumin ethanolic extract at different concentration							
			50%		40%		30%		20%	
	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)
01.	3.96×10^5	4.5×10^5	N	N	N	N	9×10^2	1.06×10^3	3.56×10^5	3.92×10^5
02.	2.68×10^5		N		N		12×10^2		4.20×10^5	
03.	6.86×10^5		N		N		11×10^2		4×10^5	

N.B. Avg. = average; SL. No. = Serial number; N = No growth, CFU = Colony Forming Unit. Each time 100µl solution was inoculated in Petri dish.

Additionally, two more concentrations of the extract (35% and 25%) were prepared to determine the antibacterial activity. At 35% concentration of ethanolic black cumin extract, not any growth of the bacteria observed (Table 05). But at the 25% concentration average load found was 2.4×10^5 that indicated a reduction of load in 46.6%.

Table 5: *V. cholerae* load before and after treatment with black cumin ethanol extract at 35% and 25% concentration.

SL. no	Without extract		With black cumin ethanolic extract at different concentration			
			35%		25%	
	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)	Load (CFU/ ml)	Avg. Load (CFU/ ml)
01.	3.96×10^5	4.5×10^5	N	N	1.2×10^5	2.4×10^5
02.	2.68×10^5		N		3.2×10^5	
03.	6.86×10^5		N		2.8×10^5	

N.B. Avg. = average; SL. No. = Serial number; N = No growth, CFU = Colony Forming Unit. Each time 100 µl solution was inoculated in Petri dish.

Process B: By determining the zone of inhibition of black cumin extract (both in acetone and ethanol) against *V. cholerae*. (Disc diffusion)

The efficacy of different concentrations of ethanolic and acetone extracts of black cumin seed against *V. cholerae* is shown in Table 7. Both extracts of black cumin seed exhibited antibacterial activity. Antibacterial activity was evaluated by measuring the zone of inhibition in mm.

It was observed in the study, with the increase of the concentration of extract from 20% to 50%, the diameters of inhibition zone (mm) of black cumin seed against the bacteria were increased. The zone of inhibition of acetonic black cumin extract ranged from 6.15±0.05 mm to 13.275± 0.025mm.

On the other side, at 20% concentration ethanolic black cumin extract, no inhibition zone was found. The zone of inhibition of ethanolic black cumin extract ranged from 5.75±0.00mm to 11.15±0.0 mm up to 50% concentration (Table 06).

These results are shown in the following table (Table 7). The result reveals that zone of inhibition produced by the both extracts of black cumin seed had a relationship with the concentration of extracts.

Process C: By determining the minimum inhibitory concentration (MIC) on the test organisms (*V. cholerae*)

The MIC of the cumin (*N. sativa*) seed extract was determined by tube dilution techniques in nutrient broth. Growth of the bacteria was assessed by recording optical density using a spectrophotometer (T 60 UV-Visible spectrophotometer). For this study the optical density was set as 600 nm because at this wavelength, absorbance of light by other molecules in the cell, such as flavins and carotenoids, is minimal (MMBB255 week 5).

Table 6: Effect of *N. sativa* in different concentrations of two extracts against *V. cholera*

Size of inhibition zone (mm) of Black cumin extract with different concentration*		
Extract Concentrations	**In Acetone (mm)**	**In Ethanol (mm)**
50%	13.275±0.025	11.15±0.00
40%	11.05±0.00	10.10±0.00
30%	13.05±0.00	9.5±0.00
25%	12.00±00	5.15±0.00
20%	6.15±0.05	N

N.B.*Value presented means of three replicates. N = No Zone, ± Standard deviation; each disc (4 mm) loaded with approximately 20 µl of black cumin extracts.

3.3.3 Determination of minimum inhibitory concentration (MIC) of the acetone extract

The MIC value of black cumin acetonic extract is shown in Table 07. The result revealed that at the concentration of 50%, 40%, 30% and 25%, bacterial growth was inhibited. So 25% (250µl/ml) was the highest dilution at which growth was minimal. So the MIC value for black cumin extract in acetone was 250µl/ml (25%).

Table 7: Minimum inhibitory concentration (MIC) of black cumin acetone extract against *V. cholerae* on nutrient broth determined by a spectrophotometer (OD_{600})

Tube no.	1	2	3	4	5	6	7	8	9
Concentration (µl/ml)	500	400	300	250	125	62.5	31.25	15.6	control
Absorbance (Abs)	0.030	0.058	0.062	0.089	0.134	0.379	0.575	0.623	0.850

3.3.4 Determination of minimum inhibitory concentration (MIC) of the ethanol extract

The MIC value of black cumin ethanolic extract is shown in Table 8.The result revealed that at the concentration of 50%, 40% and 30%, bacterial growth was inhibited. So 30% (300µl/ml) was the highest dilution at which growth was minimal. So the MIC value for black cumin extract in ethanol was 300µl/ml (30%).

Table 8: Minimum inhibitory concentration (MIC) of black cumin ethanol extract against *V. cholerae* on nutrient broth determined by a spectrophotometer (OD_{600})

Tube no.	1	2	3	4	5	6	7	8	9
Concentration (µl/ml)	500	400	300	250	125	62.5	31.25	15.6	control
Absorbance (Abs)	0.027	0.058	0.062	0.122	0.361	0.327	0.537	0.716	0.850

4. Discussion

The result of the present experiment showed that, the average *V. cholerae* load in Lottya (*H. nehereus*) and Chingri (*P. monodon*) were 3.1×10^4 CFU/g and 2.67×10^3 CFU/g. No *V. Cholerae* was found in Churi shutki (*T. haumela*). The study showed that dried fishes sold in fish market were contaminated with the pathogenic bacteria. This might be due to the unhygienic handling of the fisher folks, improper processing and unhygienic vendors and, venting area. According to WHO, 1991 [22], *V. cholerae* has been associated with consumption of numerous types of fishery products including: crustaceans (shrimp, crab, lobster), shellfish (oysters, clams, mussels, scallops, abalone) and finfish, including dried processed fish. Therefore some research said

that incidence of pathogens in the dry fish samples of fish market may be attributed to external contamination and poor sanitation in fish handling at ambient temperature [23].

The present study has found the potential antibacterial effect of the medicinal plant, black cumin (*N. sativa)* seed on the isolated human pathogenic bacteria *V. cholerae* from dried fish. Black cumin seed was chosen in this study as it is digestible to human and its tremendous health benefit as it was described earlier. From the result of inhibition zone it was seen that area of zone increased with concentration. Higher the concentration, larger the zone.

Again Mandal, *et al.,* 2014 [24] evaluated the antibacterial activity of *Mimusops elengi (M. elengi*) seed (MSE) and *Bauhinia variegate (B. variegata*) seed (BSE) extracts against *Salmonella enterica* serovar Typhi *(S. typhi*) and *V. cholerae* by agar diffusion method in ethanolic extract. The *V. cholerae* BSE (500 µg) and MSE (500 µg) had the Zone Diameter Inhibition was 12-17 mm amongst the different isolates where 13.275 ± 0.025 mm and 11.15 ± 0.0 mm zone found from the study at 50% concentration of acetonic and ethanoic extract of *N. sativa* against *V. cholerae*.

The MIC value for black cumin extract in acetone and ethanol were 250µl/ml (25%) and 300µl/ml (30%) respectively. Black cumin seed oil exhibited MIC at 0.8-3.2% against bacilli: *Escherichia coli, Salmonella enteric, Vibrio parahaemolyticus* in broth microdilution [25].

Some relevant studies has also been conducted in by Singh *et al*., (2013) [26] on 15 types of Indian spices and prepared their extract in three types of solvents (ethanol, methanol and acetone) with the concentration of 70% against three *Vibrio species (V. cholerae, V. alginolyticus, V. parahaemolyticus)* in which the extract in acetone showed maximum inhibitory effect in all fifteen different spices while minimum inhibitory effect was seen in case of ethanol. This research support the present study as acetone extract of black cumin was found more effective than ethanol.

5. Conclusion

Sometime dried fishes are easily contaminated virus and fungus. In this circumstance causes huge economic losses of fish farming. Natural seed like black cumin has the antibacterial activity which is capable to destroy virus and bacteria. Therefore black cumin extract would be a nice agent to inhibit of the growth of pathogenic bacteria.

6. References

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