Control of Some Human Pathogenic Bacteria by Seed Extracts of Cumin (*Cuminum cyminum* L.)

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Summary

Antibacterial activity of seed extracts of cumin (*Cuminum cyminum* L.) was investigated against 10 gram positive and gram negative bacteria. Disc diffusion method was used to test antibacterial activity. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by using standard procedures. The highest (effective) inhibition zone of 16.67±0.47 mm was found at 250 mg/ml for *Escherichia coli.* On the other hand, the inhibition zones 15.00±0.82 mm for ethanol, 15.33±0.47 for methanol, and 15.67±0.82 for acetone were found against *Bacillus subtilis, Sarcina lutea* and *Klebsiella pneumonia,* respectively. MIC value (20 to 50 mg/ml) and MBC value (40 to 60 mg/ml) were measured against studied bacteria. On the basis of investigation, we can say, cumin seeds could be used as a source of new antibacterial agent for developing drugs to inhibit some human pathogenic bacteria.

Key words

Cumin seed, antibacterial activity, MIC and MBC

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Introduction

Human pathogenic bacteria is one of the most serious threats to health. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobial substances (Das *et al*., 1999). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Parekh and Chanda, 2007). Spices are rich source of biologically active antimicrobial compounds and are the common dietary adjuncts that contribute to the taste and flavor of foods as well as are recognized to stabilize the foods from the microbial deterioration (Kizil and Sogut, 2003). There is extensive scientific literature on the antimicrobial potential of spices that have been reviewed by several research scientists (Lanciotti *et al*., 2004 and Sagdic, *et al*., 2003). Cumin (*Cuminum cyminum* L.) has a broad antibiotic spectrum against both gram-positive and gram negative bacteria. Cumin is an aromatic plant included in the *Apiaceae* family. It is used to flavor foods, for medical preparations, food industries and added to fragrances (Iacobellis, *et al*., 2005). In addition, Cumin is used as antispasmodic, carminative, and appetite stimulant agent (Morton, 1976). Moreover, cumin oil shows a high antifungal activity against various pathogenic fungi (Afifi, 1994; Hammad and Youssef, 1995 and Rahman *et al*., 2000). It is also used as a fumigant or additive in the storage of foodstuff (Farag *et al*., 1990 and Tunc *et al*., 2000). The present study was carried out to determine the potential antibacterial agent of ethanol, methanol and acetone seed extracts of cumin (*C. cyminum* L.) against some human pathogenic bacteria.

Materials and methods

Plant material. The Cumin seeds (*Cuminum cyminum*) were purchased from Municipal market at Kushtia, Bangladesh during February, 2008 and kept in sterilized screw-cap glass container. Samples were crashed and transferred into glass container and preserved it until extraction procedure in the laboratory.

Preparation of seed extracts. Cumin seeds were properly dried and pulverized into a coarse powder as reported by Hanafy and Hatem, 1991. Each of 20 g grinded powders was soaked in 60 ml ethanol (95 %), methanol and acetone separately for seven days with occasionally shaking. After seven days of dissolving materials were filtered through Muslin cloth into a beaker and then Whatman no. 1 filter paper were used for final filtration. Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 4^0C in air tight screw-cap tube.

Test microorganisms. Ten bacterial species, five gram negative namely *Shigella shinga, Shigella dysenteriae, Klebsiellas pneumoniae, Salmonella typhi,* and *Escherichia coli* and five gram positive namely *Sarcina lutea, Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus,* and *Streptococcus haemolytica* were used during this study. All the tested strains were supplied by International Centre for Diarrhoeal Disease Research of Bangladesh (ICDDRB), Dhaka, Bangladesh (Table 1). Bacteria were grown in Luria-Broth (LB) medium and maintained on nutrient agar slant at 4°C.

Table 1. List of bacteria used in this study

Bacteria	Accession Number
Gram negative	
Shigella dysenteriae	BMLRU1011
Shigella shinga	BMLRU1013
Escherichia coli	BMLRU1001
Klebsiellas pneumoniae	BMLRU1005
Salmonella typhi	BMLRU1009
Gram positive	
Sarcina lutea	BMLRU1012
Bacillus megaterium	BMLRU1010
Bacillus subtilis	BMLRU1008
Staphylococcus aureus	BMLRU1002
Streptococcus haemolytica	BMLRU1006

BMLRU, Biotechnology and Microbiology Laboratory of Rajshahi University

Antibacterial assay. To determine the antibacterial activity of seed, cumin seeds with ethanol, methanol and acetone extraction were used. In antibacterial screening, nutrient agar (Hi media, India) was used as culture media. Then evaluation of antibacterial activity was performed using standard agar disc diffusion method (Brooks and Orston, 2002). Filter paper was cut into small discs using a paper punch (6 mm in diameter) and impregnated with 10 µl of different concentration (250, 200, 150, 100 and 50 mg/ml) of seed extracts of *Cuminum cyminum* L.; dried off and placed on seeded agar plates. 25 µl of bacterial cultures were used for inoculation per agar plates. After inoculation and placing the disc, seeded agar plates were incubated at 37°C for 24 hours. After incubation, the diameter of zone of inhibition was measured accurately by millimeter scale. The results were compared with standard or broad-spectrum antibiotics (Tetracycline at 30 µg/ml) as a positive control. Furthermore, disc was prepared using 95 % ethanol, methanol and acetone instead of cumin seed extracts for negative control.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Standard procedure for determination of MIC and MBC of seed extracts of *Cuminum cyminum* L., were followed as previously described by Doughari, 2007. Five different concentration (60, 50, 40, 30 and 20 mg/ml) of seed extracts were used for MIC determination. 0.5 ml of varying concentration of these extracts were taken into test tubes and 2 ml nutrient broth was added separately; finally a loopful bacterial suspension was introduced in those test tubes. Tubes containing bacterial cultures were incubated at 37^0C for 24 hours. The lowest concentration of extracts that inhibit growth of selected bacteria in test tubes was taken as MIC. The test tubes containing bacterial suspension were considered as a negative control. Series test tubes from the MIC position that did not show any visible growth of bacteria were used for MBC determination. A loopful of broth was collected from tubes and streaked on nutrient agar plates. After streaking the agar plates were incubated at 37^0C for 24 hours. MBC was recorded by using the lowest concentration of extracts at which no visible growth was seen of studied bacteria on agar plates.

Results

Antibacterial assay

Ethanol, methanol and acetone extracts of *Cuminum cyminum* L. inhibited the growth of all the bacteria studied (Table 2, 3 & 4). For ethanol extract of Cumin, the highest inhibition zones (15.67±0.47 and 15.00±0.82 mm) were measured at 250 mg/ml against *Escherichia coli* and *Bacillus subtilis,* respectively, where the lowest inhibition zones (13.33±0.94 and 12.67±0.94) were measured against *Klebsiellas pneumoniae* and *Bacillus megaterium*. Methanol extract, displayed a relatively better antibacterial effect against tested bacteria. The highest inhibition zones (16.67±0.47 and 15.67±0.47 mm) were measured at 250 mg/ml against *Escherichia coli* and *Bacillus subtilis.* On the other hand, the lowest inhibition zones (13.33±0.94 and 13.00±0.94 mm) were measured against *Klebsiellas pneumoniae* and *Bacillus megaterium,* respectively. Acetone extract also showed the antibacterial effect against almost all the tested gram negative and gram positive bacteria and their highest diameter zones of inhibition were found (15.67±0.82 and 15.00±0.47 mm) against *Klebsiellas pneumoniae* and *Staphylococcus aureus*, respectively, and the lowest inhibition zones (13.67±0.47 and 13.00±0.82 mm) against *Salmonella typhi* and *Streptococcus haemolytica*, respectively. In all cases, the activity of the extracts were compared with standard antibiotic Tetracycline.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of ethanol extracts of Cumin against some pathogenic gram negative and positive bacteria ranged from 20 mg/ml to 50 mg/ml (Table 5). The highest MIC value (50 mg/ml) was found against *Shigella shinga* and *Bacillus subtilis*, whereas the lowest (20 mg/ml) against *Escherichia coli*, *Salmonella typhi*, *Sarcina lutea* and *Streptococcus haemolytica*. In case of the MIC of methanol extracts of Cumin, the highest MIC value (40 mg/ml) was recorded against *Shigella shinga, Klebsiellas pneumoniae* and *Salmonella typhi*, while the lowest (20 mg/ml) against *Shigella dysenteriae* and *Escherichia coli*. On the other hand, the gram positive bacteria had highest MIC value (40 mg/ml) against *Bacillus subtilis* and *Staphylococcus aureus* and the lowest (20 mg/ml) against *Sarcina lutea*, *Bacillus megaterium* and *Streptococcus haemolytica.* The acetone extract showed the highest MIC value (50 mg/ml) against *Escherichia coli*, whereas the lowest (20 mg/ml) against *Shigella dysenteriae* and *Klebsiellas pneumoniae*. In the case of gram positive bacteria the highest MIC value (50 mg/ml) was showed against *Sarcina*

Table 2. *In vitro* antibacterial activities of Ethanol Extract of *Cuminum cyminum* L.

Name of bacteria	Diameter of inhibition zone (mm)					
	Ethanol extract (mg/ml)					Tetracycline (µg/ml)
	250	200	150	100	50	30
Gram negative						
Shigella dysenteriae	14.00±0.82	12.00±0.82	10.33±0.38	9.00±0.82	7.67±0.94	22.00±0.58
Shigella shinga	14.33±0.47	13.33±0.47	11.67±0.19	8.67±0.47	+	30.00±0.58
Escherichia coli	15.67±0.47	14.33±0.47	12.67±0.47	10.33±0.38	8.00±0.82	27.50±0.33
Klebsiellas pneumoniae	13.33±0.94	12.67±0.94	11.33±0.19	8.00±0.82	+	28.00±0.41
Salmonella typhi	14.33±0.47	13.33±0.47	11.67±0.19	8.67±0.47	7.33±0.47	27.50±0.33
Gram positive						
Sarcina lutea	14.00±0.82	12.67±0.47	10.33±0.38	8.00±0.82	+	24.67±0.33
Bacillus megaterium	12.67±0.94	11.67±0.19	9.33±0.47	8.00±0.82	+	26.00±0.58
Bacillus subtilis	15.00±0.82	14.33±0.47	12.67±0.94	10.33±0.38	8.00±0.82	27.50±0.33
Staphylococcus aureus	13.67±0.47	12.00±0.00	10.33±0.38	8.67±0.47	7.33±0.47	27.00±0.58
Streptococcus haemolytica	14.33±0.47	12.67±0.47	9.33±0.47	8.67±0.47	+	26.33±0.67

Values are represented as mean ± S.E. of triplicate experiments, + = Growth

Table 3. *In vitro* antibacterial activities of Methanol Extract of *Cuminum cyminum* L.

Bacteria	Diameter of inhibition zone (mm)					
	Methanol extract (mg/ml)					Tetracycline (µg/ml)
	250	200	150	100	50	30
Gram negative						
Shigella dysenteriae	14.67±0.47	12.67±0.47	10.33±0.38	9.33±0.47	7.67±0.94	22.00±0.58
Shigella shinga	15.00±0.82	12.67±0.47	10.33±0.38	9.33±0.47	+	31.67±0.33
Escherichia coli	16.67±0.47	14.00±0.82	12.67±0.94	10.33±0.38	8.00±0.82	27.00±0.58
Klebsiellas pneumoniae	13.33±0.94	11.67±0.19	9.67±0.47	8.00±0.82	+	28.00±0.58
Salmonella typhi	14.00±0.82	10.33±0.38	9.33±0.47	8.00±0.82	+	28.00±0.58
Gram positive						
Sarcina lutea	15.33±0.47	12.67±0.47	10.67±0.47	8.00±0.82	7.33±0.47	24.67±0.33
Bacillus megaterium	13.00±0.82	11.67±0.19	10.33±0.38	9.33±0.47	7.67±0.94	28.00±0.58
Bacillus subtilis	15.67±0.47	14.00±0.82	12.67±0.47	9.33±0.47	+	27.00±0.58
Staphylococcus aureus	14.67±0.47	12.67±0.47	10.33±0.38	8.00±0.47	+	27.00±0.58
Streptococcus haemolytica	13.33±0.94	12.00±0.00	10.67±0.47	9.33±0.47	7.33±0.47	27.33±0.33

Values are represented as mean ± S.E. of triplicate experiments, + = Growth

Table 4. *In vitro* antibacterial activities of Acetone Extract of *Cuminum cyminum* L.

Bacteria	Diameter of inhibition zone (mm)					
	Acetone extract (mg/ml)					Tetracycline (µg/ml)
	250	200	150	100	50	30
Gram negative						
Shigella dysenteriae	14.00±0.82	12.67±0.47	10.67±0.47	9.33±0.47	7.33±0.47	22.00±0.58
Shigella shinga	14.33±0.47	11.67±0.19	10.67±0.47	8.67±0.47	+	31.67±0.33
Escherichia coli	13.67±0.47	11.67±0.19	9.67±0.47	8.00±0.47	+	27.00±0.58
Klebsiella pneumoniae	15.67±0.82	14.33±0.47	12.33±0.94	9.33±0.47	8.00±0.82	28.00±0.58
Salmonella typhi	13.67±0.47	11.67±0.19	9.67±0.47	8.00±0.47	+	28.00±0.58
Gram positive						
Sarcina lutea	13.33±0.94	12.00±0.00	9.33±0.47	8.00±0.47	+	24.67±0.33
Bacillus megaterium	14.00±0.82	10.33±0.38	9.33±0.47	8.00±0.82	+	28.00±0.58
Bacillus subtilis	14.33±0.47	11.67±0.19	10.67±0.47	8.67±0.47	7.67±0.94	27.00±0.58
Staphylococcus aureus	15.00±0.47	11.67±0.19	9.67±0.47	8.00±0.47	+	27.00±0.58
Streptococcus haemolytica	13.00±0.82	11.67±0.19	10.33±0.38	9.33±0.47	7.67±0.94	27.33±0.33

Values are represented as mean ± S.E. of triplicate experiments, + = Growth

Table 5. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of ethanol, methanol and acetone seed extracts of *Cuminum cyminum* L. against some human pathogenic bacteria.

Bacteria	EE (mg/ml)		ME (mg/ml)		AC (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
Gram negative						
Shigella dysenteriae	30	60	20	40	30	50
Shigella shinga	50	60	40	50	50	50
Escherichia coli	20	40	20	40	50	60
Klebsiellas pneumoniae	40	50	40	60	30	50
Salmonella typhi	20	40	40	60	40	60
Gram positive						
Sarcina lutea	20	40	20	40	50	60
Bacillus megaterium	40	50	20	50	40	50
Bacillus subtilis	50	60	40	60	30	40
Staphylococcus aureus	40	50	40	60	40	60
Streptococcus haemolytica	20	40	20	50	30	50

Values are represented as mean; EE= Ethanol Extract, ME=Methanol Extract and AC= Acetone Extract

lutea, while the lowest (20 mg/ml) against *Bacillus subtilis* and *Streptococcus haemolytica*. Moreover, in ethanol extracts, the highest MBC value (60mg/ml) was found against *Shigella dysenteriae* and *Shigella shinga* while the lowest (40 mg/ml) against *Escherichia coli* and *Salmonella typhi*. On the other hand, the highest MBC value (60 mg/ml) was in case the of the gram positive bacteria against *Bacillus subtilis* and the lowest (40 mg/ml) against *Sarcina lutea* and *Streptococcus haemolytica*. The highest MBC value (60mg/ml) for negative bacteria was recorded against *Klebsiellas pneumonia* and *Salmonella typhi* and the lowest (40 mg/ml) against *Shigella dysenteriae* and *Escherichia coli* in menthol extraction. The highest MBC value (60 mg/ml) was found against *Bacillus subtilis* and *Staphylococcus aureus* and the lowest (40 mg/ml) against *Sarcina lutea*. In acetone extract, the highest MBC value (60mg/ml) was observed against *Escherichia coli* and *Salmonella typhi* and the lowest (50 mg/ml) against *Shigella dysenteriae, Shigella shinga* and *Klebsiella pneumoniae* for gram negative bacteria. On the other hand, for gram positive bacteria, the highest MBC value (60 mg/ml) was measured against *Sarcina lutea* and *Staphylococcus aureus* and the lowest (40 mg/ml) against *Bacillus subtilis*.

Based on measurement of zone of inhibition, MIC and MBC determination proved that the most susceptible bacteria, namely *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae* and *Staphylococcus aureus* might be controlled with the cumin seed extracts.

Discussion

The results showed that the methanol extract of *Cuminum cyminum* L. seeds had the best antimicrobial activity. Most of the highest activity against gram negative bacteria *E. coli* showed inhibition zone- 16.67±0.47 mm while the nearest activity- 15.00±0.82, 15.67±0.82, and 14.67±0.47 mm were recorded on *S. shinga, K. pneumoniae,* and *S. dysenteriae,* respectively. Gram-negative bacteria have been found to be less sensible to plant extracts.

This result is similar to previous researcher's results (Afolayan and Meyer, 1995 and Kuhnt, 1995). The inhibition zones recorded were 14.00±0.82, 13.00±0.82, 12.67±0.94 and 13.67±0.47 against *S. lutea*, *S. haemolytica, B. megaterium*, *and S. aureus,* respectively. MIC and MBC were determined using standard methods (Doughari, 2007). MIC value (ranging 20-50 mg/ml) and MBC value (ranging 40–60 mg/ml) were recorded against all the studied bacteria. The highest MIC and MBC values suggest that the seed extracts are less susceptible. Recently, multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs that are commonly used in the treatment of infectious diseases, making it a global growing problem (Parekh and Chanda, 2007). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Antimicrobial characteristics of the herbs are due to various chemical compounds including volatile oils, alkaloids, tannins and lipids that are presented in their tissue (Baytop, 1984 and Con *et al*., 1998). Preliminary clinical trials have documented its therapeutic use for the treatment of variety of diseases and conditions that include diarrhea, asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever,

dizziness, influenza and dental caries (Ali and Blunden, 2003 and Gilani *et al*., 2001). In addition, different pharmacological effects such as insulinotropic, hypoglycemic, anti-cancer, anti-nociceptive, antiinflammatory, hepatoprotective, neuroprotective, antihistaminic, antiulcer and bronchodilatory activities have been reported for Kalonji (Hosseinzadeh *et al*., 2007). Spices are frequently used as an active ingredient in certain medicines and reported to possess a number of pharmacological effects to treat different human ailments (Bonjar, 2004). Several investigations have been directed towards their antibacterial properties (Voravuthikunchai *et al*., 2005). Previous research studies have documented that *E. coli* are known to be multi-drug resistant (Saeed *et al*., 2007 and Singh *et al*., 2002). Present study is also in agreement with Morsi's (2000) results who reported that Kalonji extracts showed antibacterial activity against a broad range of microbes and including multiple antibiotic resistant bacteria. In a recent survey, pharmacological studies have been conducted on the ethanol, methanol and acetone extracts of defatted *Cuminum cyminum* L. seeds to evaluate their effects on the central nervous system and on analgesic activity. Besides, cumin is one of the popular spices regularly used as a flavoring agent in a number of ethnic cousins. In Iranian ancient medicine, the fruits of the plant have been used for the treatment of toothache, diarrhea and epilepsy. Some researchers noted that cumin as an emerging alternative antimicrobial agent for human applications (Janahmadi *et al*., 2005). Thus according to our investigation Cumin has antibacterial potential and can be used as a potent antibacterial agent for human pathogenic bacteria.

Conclusion

The cumin seed has the important antimicrobial activity against the tested strains. In this regard, the use of spice and their volatile compounds as natural preservatives in food products may be an alternative to the use of chemical additives. Our results also indicated that extract of the cumin seed with methanol, has a strong inhibitory activity on some pathogens.

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