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Research Article
Biological Control of Foodborne Pathogenic *Enterobacter* sp. and *Bacillus* sp. Using Different Spice Extracts Available in Bangladesh

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Abstract
Background and Objective: The relatively inexpensive, widely available poultry meat and eggs can be of central importance to meet shortfalls in essential nutrients, particularly to impoverished people. Contamination of foods by pathogenic microbes causes consumer illness. In this study, antibacterial activity of spice extracts against isolated foodborne pathogenic bacterial strains were evaluated. **Materials and Methods:** Egg and meat samples were collected from poultry egg and meat retailers of local market and screened on MacConkey and Mannitol salt agar media, respectively. The isolates were identified by morphological, biochemical and 16S rRNA gene sequence analysis. Anti-bacterial activity of different spice extracts against isolated bacteria was tested through disc diffusion method. Cytotoxicity was evaluated through probit mortality analysis software. **Results:** *Bacillus* sp. (from meat) showed more toxicity (LC_{50} = 95.6205 µL mL^{-1}) than *Enterobacter* sp. (from egg) (LC_{50} = 143.2504 µL mL^{-1}) against *Artemia salina*. For biological control, methanolic and aqueous extracts of *Cinnamomum verum*, *Syzygium aromaticum*, *Piper chaba*, *Zingiber officinale* were used. Methanolic extract of *S. aromaticum* acted as potential antimicrobial agent against *Bacillus* sp. with a inhibition zones of 15$\pm$0, 18.33$\pm$0.58 and 21$\pm$0 mm at a concentration of 50, 75, 100 µg mL^{-1}, respectively. Similarly, *Enterobacter* sp. was susceptible at the same doses with a inhibition zones of 15.33$\pm$0.58, 16.67$\pm$0.58 and 18$\pm$0 mm. Moreover, *C. verum* oil with a dose of 30 µL showed the highest growth inhibitory effect against *Bacillus* sp. (inhibition zone 40$\pm$1 mm) than *Enterobacter* sp. (inhibition zone 34.33$\pm$1.53 mm). **Conclusion:** The result suggests that spices represent an alternative source of natural antibacterial substances for inhibiting the growth of foodborne bacteria.

Key words: Microbial pathogens, LC_{50}, agar well diffusion methods, antimicrobial susceptibility, spice extracts

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Foods, by their nature are nutritious, easily metabolisable and therefore offer suitable substrates for the growth and metabolism of microorganisms. Foodborne diseases are a serious concern as public health issue particularly in developing countries[1]. Unsafe foods are responsible for many foodborne and waterborne diarrhoeal illnesses which kills an estimated 2.2 million people every year globally, most of them are children[2]. Microorganisms are the primary cause of food spoilage and food borne illness. Many different sources like bacteria, viruses, parasites, chemicals and poison may be transmitted to humans by contaminated food. Approximately 175 pathogenic species are associated with diseases considered to be emerging and approximately 54% of emerging infectious diseases are caused by bacteria, among which 30% are transmitted through food[3]. Outbreaks and sporadic cases of food borne disease are regular occurrences in all countries of the world[4]. Generally, food borne diseases are associated with acute, mild and self-limiting gastroenteritis with symptoms such as nausea, vomiting, diarrhoea and may affect the cardiovascular, musculoskeletal, respiratory and immune systems[5]. The emergence of these foodborne illness-causing bacteria is largely related to consumption of poultry meat or eggs.

Eggs are one of the most important foods of animal origin which contain many nutrients as well as the availability, modest cost, ease of preparation, popular taste appeal and low caloric value give eggs a deserved place in the diets especially in children diet[6]. During collection, washing, sorting, transportation and packaging of eggs it can be contaminated, re-contaminated and cross contaminated by some vehicles like fomites, flies, dust, faces and rodents[7]. Microbial contamination of egg shells is an increasing concern to the farmers and consumers of hens' eggs and poultry products in general[8]. *Citrobacter youngae*, *Enterobacter cloacae*, *Escherichia coli*, *Flavimonas oryzihabitans*, *Klebshiella pneumoniae*, *Proteus* sp., *Salmonella* sp., *Serratia* sp., *Shigella* sp., *Xanthomonas maltophilia* and *Yersinia* sp. are some identified isolates in eggs and on shells[9].

Though meat is an important source of proteins, essential fatty acids, minerals and vitamins but it can act as a suitable medium for the growth of various microorganisms[10]. Meat can be easily contaminated during bleeding, handling and processing via knives, tools, clothes, hands and air[11]. The contaminated meat and meat products readily cause a variety of biological, chemical, physical and particularly microbial food hazards[12]. The most important foodborne bacterial pathogens associated with meat are *Salmonella* sp., *Staphylococcus aureus*, *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Clostridium perfringens*, *Yersinia enterocolitica* and *Bacillus cereus*[13].

Such growing concern about food safety has led to the development of natural antimicrobials[14]. Spices have been used as antimicrobial agents since ancient times[15] and their essential oils, alone or in combination have also been used to test the activity against different microbes[16]. In our country, clove, cinnamon, choi, ginger are some of the major as well as noteworthy traditional spices which have been given special attention to find their antibacterial activity. Cloves are used as a carminative to increase hydrochloric acid in the stomach and to improve peristalsis. Cinnamon is high in antioxidant, relieves inflammation and protects heart health. The crude extract of choi possess antibacterial, carminative, expectorant and smooth muscle relaxant properties. Ginger extracts have been extensively studied for a broad range of biological activities including anti-bacterial, anti-convulsant, analgesic, anti-ulcer, gastric anti-secretory, anti-tumor, anti-fungal, hypocholesterolemic, antiallergic and other beneficial activities[17].

Thus the present study was designed to isolate, characterize, biological control of pathogenic bacteria from probable food samples with special reference to their cytotoxic activity.

MATERIALS AND METHODS

Sample collection: Egg and meat samples were obtained from poultry egg and meat retailers of local market of katakhali, Rajshahi, Bangladesh. Samples were collected aseptically in sterile pack and transported to the Microbiology Laboratory, Department of Genetic Engineering and Biotechnology, Rajshahi University for further experiment. All the samples were processed within 6 h of its collection.

Isolation and growth optimization of pathogenic bacterial strains: In the present study, peptone water (peptone 10.0 g, sodium chloride 5.0 g) was used as a non-selective enrichment media for sample inoculation. For the isolation of bacteria from the surface of the egg shell, cotton swab stick was moistened in 0.1% peptone water and used to swab the surface of the egg shell and was transferred to the peptone water for incubating at 37°C for 24 h. Approximately 5 g of meat (from thigh muscle) was excised from collected sample, minced and placed in 100 mL of peptone water as non selective pre-enrichment media for incubating at 37°C for 24 h. After the non-selective enrichment, MacConkey and Mannitol agar media were used as selective media for the

isolation of Gram-negative and Gram-positive pathogenic bacterial strains, respectively from enriched food samples. Inoculated plates were incubated aerobically at 37°C and growth were examined after 18-24 h of incubation. The single colonies from the selective media were sub cultured and eventually transferred into LB agar plates. Then the plates were incubated at 37°C for 24 h and a single bacterial colony was isolated for further study.

Different pH (5, 6, 7 and 8) and temperature (25, 30, 35 and 40°C) were used for determining the optimum bacterial growth. Bacterial cell density was measured at 660 nm using double beam spectrophotometer (ANALYTK JENA AG, SPOKOL 1500/1, GERMANY. All the experiments were done in triplate.

Morphological and biochemical characterization of isolated bacteria: Bacterial isolates were characterized by several morphological and biochemical tests like gram staining[18], motility[19], methyl red[20], simmons citrate[21], catalase[22], urease[23], triple sugar iron[24], starch agar[25] tests etc.

Molecular identification of the bacterial isolates: Molecular identification has been used as a worldwide acceptable technique to identify any bacterium up to genus level across all major phyla. At first, the genomic DNA of the isolated bacterial strains was extracted (about 1465 bp) using phenol/chloroform method[26]. The 16S rRNA genes were amplified by PCR using 16S rRNA specific primer forward primer 27F 5-AGAGTTTGATCMTGGCTCAG-3 and reverse primer 1492R 5 -GGTTACCTTGTTACGACTT-3. The PCR reactions were carried out in thermal cycler (Applied Biosystem 9700, USA) using following amplification conditions: An initial denaturation step at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and the final extension at 72°C for 10 min. The PCR products were purified and were sequenced for both strands on genetic analyzer (Prism 310, USA). The sequences were then edited by bioinformatics software Chromas. The homology of the 16S rRNA gene sequences were checked with the 16S rRNA gene sequences of other organisms using the BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi) algorithm.

Cytotoxicity evaluation of isolated bacterial strains: Cytotoxic activities of bacterial strains were evaluated through brine shrimp (*A. salina*) lethality assay[27] at different concentrations of 50, 100, 150 and 200 µL. The mortality (%) of the brine shrimp nauplii was calculated for every concentration for each sample. A plot of log concentration of the sample dose levels versus percent of mortality showed an approximate linear correlation between them.

Antibacterial activity test: In this study, the antibacterial screening of spice extracts (methanolic and aqueous) were undertaken with well diffusion method[28] as well as *C. verum* (Cinnamon) oil with disc diffusion method[29].

Spice extracts preparation using methanol and aqueous solvent: The fresh spice samples (*Cinnamomum verum, Syzygium aromaticum*, *Piper chaba*, *Zingiber officinale*) and *C. verum* (Cinnamon) oil used in the present study were obtained from the local market of Rajshahi during August, 2017. The collected spice samples were washed, surface sterilized with 0.1% $HgCl_2$, unwanted materials were discarded and were dried properly in room temperature. The dried spices were grinded into small fine particles by a grinder machine, the powder was transferred to an air tight container and kept in a cool and dark place until the screening of their bioactive potentialities. For extraction, about 10 g powdered of each material was taken in 1 L conical flasks. Each sample was soaked in 50-60 mL of methanol and water. The conical flasks with its contents were then sealed and kept on orbital shaker for continuous shaking at 150 rpm for 2 days. The mixtures were then filtered through Whatman No.1 filter paper. Using rotary evaporator the methanolic and aqueous extract of each spice was evaporated at 55-60°C temperatures at 160-180 rpm. After 30 min of drying process, a slurry concentration was obtained, which was kept in small vial for further drying. After 20-30 days, the solvents were completely evaporated and the extracts became ready for experiment. In case of antibacterial activity, 5 mg of each extract was taken in separate vials and 5 mL of solvent (methanol and water) were carefully added in each vial. The extracts were then dissolved well in the solvent by inverting the tube. Each of the stock solution with concentration of 1 $\mu g\ \mu L^{-1}$ was labeled and used for sensitivity test.

Antibacterial screening of spice extracts: Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts[30]. Nutrient agar was prepared according to the manufacturer's instructions, autoclaved and dispensed into sterile petri dishes and allowed to set before use. Usually, 20 mL nutrient agar was placed into 10 mL petri dishes and 0.1 mL of the active bacterial cultures were spread over the plate using a sterile glass spreader in order to get a uniform microbial growth for all plates. A well was done using

a 8 mm diameter cork borer in the agar plate. The wells were filled with 25, 50, 75 and 100 μg mL^{-1} specific spice extracts (aqueous and methanolic). The plates were left for 30 min at room temperature to allow the diffusion of extracts. Then the plates were incubated at 37°C for 24 h. After 24 h, the inhibition zones were measured using millimeter scale. Values were performed in triplicate and the mean value was recorded. In this case, the antibiotic gentamicin (10 μg $disc^{-1}$) was used as control.

Antibacterial screening of *C. verum* oil: The discs (6 mm diameters) were made by punching the Whatman No. 1 filter paper. For the testing of the antibacterial efficiency of *C. verum* oil against one bacterial sample, paper discs were soaked with different concentrations of oil (10, 20 and 30 μL). In the present study, laboratory supplied antibiotic gentamicin (10 μg $disc^{-1}$) was used as standard. By means of a pair of sterile forceps, the dried discs and standard disc (gentamicin) were placed gently on the solidified agar plates seeded with the tested bacteria to ensure contact with the medium. The plates were then kept in an incubator at 37.5°C temperature for 24 h. After incubation, the antibacterial activities of the tested samples were determined by measuring the diameter of inhibitory zones with a millimeter scale.

RESULTS

Isolation and optimization of growth characteristics: Pathogenic bacterial strains were isolated from egg and meat sample through screening on MacConkey and Mannitol salt agar media respectively and the results are shown in Table 1. The optimum pH and temperature for the growth of both isolates was 7.0 (Fig.1a, b) and 35°C (Fig. 2a, b).

Morphological and biochemical characterization of isolated bacteria: Morphologically both the isolates were motile and rod shaped but isolate E1 was Gram-negative while isolate M2 was Gram-positive. The biochemical tests results are shown in Table 2.

Molecular identification of the bacterial isolates: After gene sequencing, the sequences were checked against the 16S rRNA gene sequences of other organisms that had already been submitted to NCBI Genebank database. Isolate E1 showed 98% identity with *Enterobacter* sp., while isolate M2 showed 93% identity with *Bacillus* sp. The band of PCR products of isolated bacteria was shown in Fig. 3.

Brine shrimp lethality assay: The present study explored the cytotoxic effect of isolated bacteria at concentrations of 50, 100, 150 and 200 μL. The LC_{50} value for *Enterobacter* sp. and *Bacillus* sp. was 143.2504 μL mL^{-1} (Fig. 4a) and 95.6205 μL mL^{-1} (Fig. 4b), respectively.

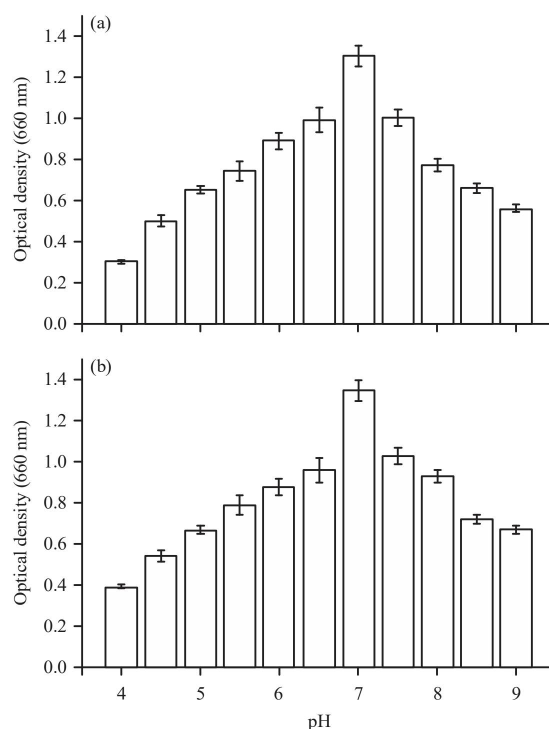

Fig. 1(a-b): Effect of pH on growth characteristics of isolate (a) E1 and (b) M2
Data were recorded after 24 h of incubation

Table 1: Microscopic and macroscopic morphological observation of bacterial isolates

	Results	
Test name	Isolate E1	Isolate M2
Gram staining	Negative	Positive
Shape	Straight rods	Rod shaped, straight, round-ended or square-ended rods
Size	0.6-1.0 μm by 1.2-3.0 μm	0.5-1.2 μm by 2.5-10.0 μm
Colour	Pink	Pink
Motility	Motile	Motile
Colonial appearance	Shiny, creamy white, smooth, irregularly round to rough colonies	White, dry, flat and irregular with lobate margins

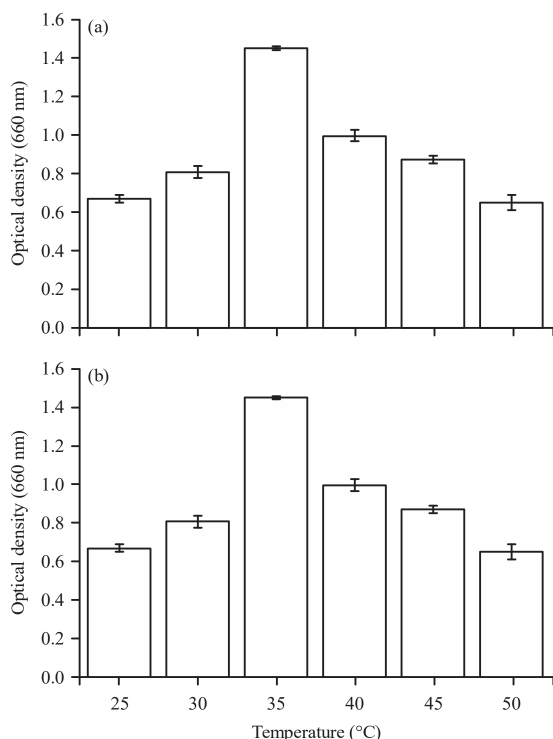

Fig. 2(a-b): Effect of temperature on growth characteristics of isolate (a) E1 and (b) M2

Data were recorded after 24 h of incubation

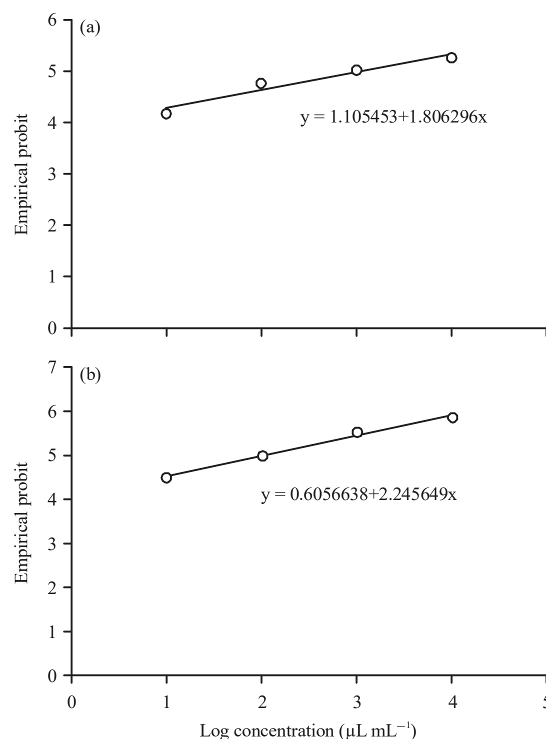

Fig. 4(a-b): Regression line and LC_{50} value of isolate (a) E1 and (b) M2

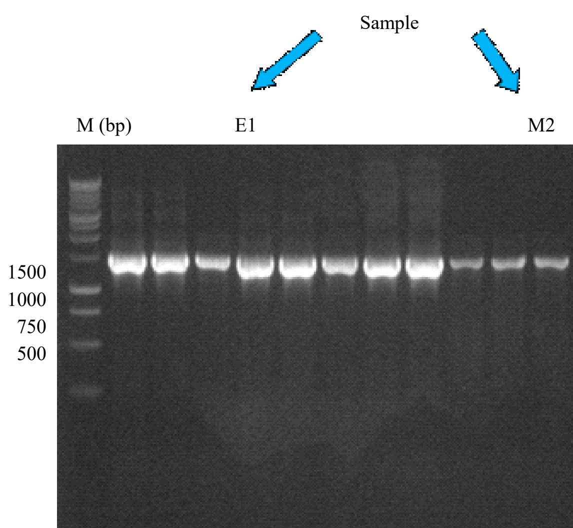

Fig. 3: Band of PCR products from isolated bacterial strains

"M": 1 kb DNA ladder (Marker), E1, M2: PCR products of isolated bacterial sample

Antibacterial activity of spice extracts: All the spice extracts (methanolic and aqueous) showed their antibacterial activity against the isolated foodborne bacterial pathogens with 4 different doses like 25, 50, 75 and 100 µg mL^{-1}.

Antibacterial activity of methanolic spice extracts: *Enterobacter* sp. was susceptible at doses of 75 and 100 µg mL^{-1} while *Bacillus* sp. was susceptible to the concentration of 100 µg mL^{-1} in case of *C. verum* methanolic extract (Table 3). For *S. aromaticum* methanolic extract, both *Enterobacter* sp. and *Bacillus* sp. were susceptible at 50, 75 and 100 µg mL^{-1} (Table 3). During the study of antibacterial activity of *P. chaba* methanolic extract against the isolated bacterial strains, *Enterobacter* sp. was susceptible at a doses of 75, 100 µg mL^{-1} as *Bacillus* sp. was susceptible at a dose of 100 µg mL^{-1} (Table 3). In case of *Zingiber officinale*, *Enterobacter* sp. was susceptible at doses of 75 and 100 µg mL^{-1} but no susceptible zones of inhibition was found against *Bacillus* sp. (Table 3).

Antibacterial activity of aqueous spice extracts: In case of *C. verum*, *P. chaba* and *Z. officinale* aqueous extract, both *Enterobacter* sp. and *Bacillus* sp. were resistant at the prospective doses (Table 4). But, in case of *S. aromaticum* aqueous extract isolated bacteria were intermediate resistant at dose 25 µg mL^{-1} and were susceptible to the remaining doses (Table 4).

Table 2: Summarized results of biochemical tests used for the characterization of the isolates

	Results	
Test name	Isolate E1	Isolate M2
Catalase test	Positive	Positive
Oxidase test	Negative	Positive
Sulfur indole motility test	Indole negative, H_2S negative, motile	Indole negative, H_2S negative, motile
Methyl red test	Positive	Negative
Triple sugar iron agar test	Positive (Glucose, sucrose, lactose fermentation with gas formation)	Positive (only glucose fermenter but no H_2S, no gas formation)
Simmons citrate agar test	Positive	Positive
Urease test	Positive	Negative
Starch agar test	Negative	Positive
MacConkey agar test	Positive (lactose fermenter)	Negative
Mannitol agar test	Negative	Positive (mannitol non-fermenter)
Eosin methylene blue agar test	Positive	Negative
Bismuth sulfite agar test	Positive	Negative

Table 3: Antibacterial activity test of methanolic extracts of different spices against the isolated bacterial strains

		Zone of inhibition (mm)		Resistant pattern	
Methanolic extracts	Dose (μg mL^{-1})	*Enterobacter* sp.	*Bacillus* sp.	*Enterobacter* sp.	*Bacillus* sp.
C. verum	25	11.00±0	12.33±0.58	Resistant	Resistant
	50	12.00±0	13.00±0	Resistant	Intermediate resistant
	75	14.33±0.58	14.00±1	Susceptible	Intermediate resistant
	100	15.67±0.58	15.33±0.58	Susceptible	Susceptible
S. aromaticum	25	13.00±0	13.00±0	Intermediate resistant	Intermediate resistant
	50	15.33±0.58	15.00±0	Susceptible	Susceptible
	75	16.67±0.58	18.33±0.58	Susceptible	Susceptible
	100	18.00±0	21.00±0	Susceptible	Susceptible
P. chaba	25	13.00±0	11.00±0	Intermediate resistant	Resistant
	50	13.67±0.58	12.67±0.58	Intermediate resistant	Resistant
	75	15.00±0	14.00±0	Susceptible	Intermediate resistant
	100	16.00±1	15.33±0.58	Susceptible	Susceptible
Z. officinale	25	13.00±0	8.00±0	Intermediate resistant	Resistant
	50	15.00±0	8.00±0	Susceptible	Resistant
	75	16.00±0	8.00±0	Susceptible	Resistant
	100	17.00±0	8.00±0	Susceptible	Resistant
Gentamicin	10	17.00±0	17.00±0	Susceptible	Susceptible

Table 4: Antibacterial activity test of aqueous extracts of different spices against the isolated bacterial strains

		Zone of inhibition (mm)		Resistant pattern	
Aqueous extracts	Dose (μg mL^{-1})	*Enterobacter* sp.	*Bacillus* sp.	*Enterobacter* sp.	*Bacillus* sp.
C. verum	25	8.67±0.58	8.00±0	Resistant	Resistant
	50	10.00±0	8.00±0	Resistant	Resistant
	75	10.67±0.58	8.00±0	Resistant	Resistant
	100	12.00±1	8.00±0	Resistant	Resistant
S. aromaticum	25	13.00±0	13.00±0	Intermediate resistant	Intermediate resistant
	50	14.33±0.58	15.33±0.58	Susceptible	Susceptible
	75	15.67±0.58	16.33±0.58	Susceptible	Susceptible
	100	16.00±1	19.00±1	Susceptible	Susceptible
P. chaba	25	8.00±0	8.00±0	Resistant	Resistant
	50	8.00±0	9.33±0.58	Resistant	Resistant
	75	8.33±0.58	11.33±0.58	Resistant	Resistant
	100	9.00±0	12.00±0	Resistant	Resistant
Z. officinale	25	8.00±0	8.00±0	Resistant	Resistant
	50	8.00±0	8.00±0	Resistant	Resistant
	75	8.00±0	8.00±0	Resistant	Resistant
	100	8.00±0	8.00±0	Resistant	Resistant
Gentamicin	10	17.00±0	17.00±0	Susceptible	Susceptible

Resistant = <13 mm, Intermediate resistant = 13-14 mm, Susceptible = >14 mm

Table 5: Antibacterial activity test of *C. verum* oil against the isolated bacterial strains

Extracts	Dose (µL mL^{-1})	Zone of inhibition (mm)		Resistant pattern	
		Enterobacter sp.	*Bacillus* sp.	*Enterobacter* sp.	*Bacillus* sp.
C. verum	10	24.33±0.58	25±1	Susceptible	Susceptible
	20	26.67±0.58	30±0	Susceptible	Susceptible
	30	34.33±1.53	40±1	Susceptible	Susceptible
Gentamicin	10	17.00±0.00	17±0	Susceptible	Susceptible

Antibacterial activity of *C. verum* oil: When the antibacterial activity of *C. verum* oil was tested, both bacterial isolates were found to be susceptible (Table 5) at all the three doses (10, 20 and 30 µL).

DISCUSSION

In this study, pathogenic bacteria were isolated and characterized from food samples (poultry eggs and meat) collected from local market of Rajshahi district, Bangladesh. Several workers have isolated pathogenic bacteria from different food sources. Jambalang *et al.*[31] isolated *Enterobacter cloacae* from retailed poultry eggs. Fayaz *et al.*[32] isolated and characterized enterotoxigenic *Bacillus cereus* from poultry meat. Optimum pH and temperature for the bacterial growth was 7 and temperatures 35°C, respectively as reported earlier by Frazier and Westhoff[33]. Molecular identification confirmed that isolate E1 had 98% similarity with *Enterobacter* sp. while isolate M2 had 93% similarity with *Bacillus* sp. All the morphological, physiological and biochemical characteristics were found similar with the respective genus for the bacterial isolates according to Bergey's Manual of Systematic Bacteriology. Through cytotoxicity test it was revealed that, *Bacillus* sp. (LC_{50} 95.6205 µL mL^{-1}) showed more toxicity than *Enterobacter* sp. (LC_{50} 143.2504 µL mL^{-1}).

Spices have been shown to possess medicinal value, in particular, antimicrobial activity. The methanolic and aqueous extracts from four traditionally used spices *Cinnamomum verum*, *Syzygium aromaticum*, *Piper chaba*, *Zingiber officinale* were prepared and evaluated for their antibacterial activity against isolated foodborne bacterial strains. Puangpronpitag and Sittiwet[34] reported antibacterial activity of *Cinnamomum verum* stem bark aqueous extract against *Straphylococcus epidermidis, Krebsilla pneumonia* and *E. coli* at 250 µg mL^{-1}. In this study, *Enterobacter* sp. was susceptible to *C. verum* methanolic extract with zones of inhibition ranging from 14.33±0.58 at concentration of 75 µg mL^{-1} to 15.67±0.58 mm at 100 µg mL^{-1} while *Bacillus* sp. was only susceptible at 100 µg mL^{-1} with 15.33±0.58 zone of inhibition. In case of *C. verum* aqueous extract, no susceptible zone of inhibition was found against the isolated bacteria at prospective doses which had similarity with the referenced data. In terms of *S. aromaticum* methanolic extract, *Enterobacter* sp. was susceptible with 15.33±0.58, 16.67±0.58 and 18±0 mm zones of inhibition at doses of 50, 75 and 100 µg mL^{-1}, respectively. Similarly, *Bacillus* sp. showed susceptibility at doses of 50, 75 and 100 µg mL^{-1} with 15±0, 18.33±0.58 and 21±0 mm zones of inhibition, respectively. In case of *S. aromaticum* aqueous extract, *Enterobacter* sp., was susceptible with 14.33±0.58, 15.67±0.58 and 16±1 mm zones of inhibition while susceptible zones of inhibition were 15.33±0.5, 16.33±0.58, 19±1 mm for *Bacillus* sp., at 50, 75 and 100 µg mL^{-1} concentration followed by the investigation of Saeed *et al.*[35]. In their study, bactericidal effect of methanolic extract of *Syzygium aromaticum* was found with 17 mm zone of inhibition and aqueous extract with 16 mm zone of inhibition against *Bacillus subtilis* at dose of 250 µg mL^{-1}. According to Roy *et al.*[36] antibacterial activity of methanolic extract of *P. chaba* stem and leaf was observed against some pathogenic bacteria at dose of 400 µg $disc^{-1}$. While studying the antibacterial activity of *P. chaba* methanolic extracts, *Enterobacter* sp. was susceptible at doses of 75 and 100 µg mL^{-1} and zones of inhibition were 15±0 and 16±1 mm, respectively but *Bacillus* sp. was only susceptible at dose of 100 µg mL^{-1} with 15.33±0.58 mm zone of inhibition. On the other hand, *Enterobacter* sp. and *Bacillus* sp. were resistant to *P. chaba* aqueous extract at applied 4 doses. Yassen and Ibrahim[37] studied anti-bacterial effect of methanolic and aqueous extracts of root of *Zingiber officinale* against *Escherichia coli* and *Staphylococcus aureus* at dose of 50 µL. In this study, *Enterobacter* sp. was susceptible to *Zingiber officinale* methanolic extract with 15±0, 16±0 and 17±0 mm inhibition zones at doses of 50, 75 and 100 µg mL^{-1}, respectively whereas, *Bacillus* sp. was resistant at respective 4 doses. However, both *Enterobacter* sp. and *Bacillus* sp. were resistant to *Zingiber officinale* aqueous extract at applied four doses which showed equal (average 8.00 mm) diameter of inhibition zone that means diameter of inhibition zone were not increased due to the increasing of dose from 25-100 µg mL^{-1}.

This research also studied anti-bacterial activity of *C. verum* oil against isolated bacteria. In case of *Bacillus* sp., the highest diameter of inhibition zone was 40±1 mm at the dose of 30 µL mL^{-1} and the other 2 inhibition zones were of 25±1, 30±0 mm at the doses of 10 and 20 µL mL^{-1}. The *C. verum* oil produced, inhibition zones of 24.33±0.58, 26.67±0.58, 34.33±1.53 mm at the doses of 10, 20 and 30 µL mL^{-1}, respectively in case of *Enterobacter* sp. which had similarity with the findings of Gupta *et al.*[38]. They investigated that *C. verum* oil exerted had strongest effect on *B. cereus* among ten pathogenic bacteria tested and also reported that *C. verum* oil was more effective than *C. verum.* In all cases, standard antibiotic Gentamicin (10 µg disc^{-1}) showed susceptibility with average 17 mm zone of inhibition.

Among different spices extract, *Syzygium aromaticum* (clove) and *C. verum* (cinnamon) methanolic extracts were found to possess relatively higher antimicrobial activities than aqueous extracts against isolated bacteria. It was also found that *C. verum* oil had highest antimicrobial effect than methanolic and aqueous spice extracts.

CONCLUSION

These results suggest that spices represent an alternative source of natural antimicrobial substances and can be used to prevent the growth of foodborne bacteria and extend the shelf-life of the processed food. However, further analysis could be done to isolate the antimicrobial agents present in these spices and to determine their minimal inhibitory concentrations so that they can be used as bio preservatives in various food stuffs.

SIGNIFICANT STATEMENT

Nutritional value, availability, low price of eggs and meat are well known. Contamination by pathogenic microbes has detrimental effects on human health. This result will help to control those bacteria by using spices extracts in a natural way. The result suggests that spices represent an alternative source of natural antimicrobial substances for inhibiting the growth of foodborne bacteria.

REFERENCES

1. Manoj, J., M.K. Singh and Y.P. Singh, 2015. The role of poultry in food borne salmonellosis and its public health importance. Adv. Anim. Vet. Sci., 3: 485-490.
2. Nata, M., 2015. Message from WHO representative to India on world health day. World Health Organization, India.
3. Oyarzabal, O.A., 2012. Emerging and Reemerging Foodborne Pathogens. In: Microbial Food Safety: An Introduction, Oyarzabal, O.A. and S. Backert (Eds.)., Springer, New York, USA., pp: 3-12.
4. Kirk, M.D., S.M. Pires, R.E. Black, M. Caipo and J.A. Crump *et al.*, 2015. World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal and viral diseases, 2010: A data synthesis. PLoS Med., Vol. 12. 10.1371/journal.pmed.1001921.
5. Tan, L.T.H., K.G Chan and L.H. Lee, 2014. Application of bacteriophage in biocontrol of major foodborne bacterial pathogens. J. Mol. Biol. Mol. Imaging, Vol. 1.
6. Layman, D.K. and N.R. Rodriguez, 2009. Egg protein as a source of power, strength and energy. Nutr. Today, 44: 43-48.
7. Kornacki, J.L. and J.L. Johnson, 2001. Enterobacteriaceae, Coliforms and *Escherichia coli* as Quality and Safety Indicators. In: Compendium of Methods for the Microbiological Examination of Foods, Downes, F.P. and K. Ito (Eds.). 4th Edn., American Public Health Association, Washington, DC., USA., pp: 69-82.
8. Knape, K.D., C. Chavez, R.P. Burgess, C.D. Coufal and J.B. Carey, 2002. Comparison of eggshell surface microbial populations for in-line and off-line commercial egg processing facilities. Poult. Sci., 81: 695-698.
9. Musgrove, M.T., D.R. Jones, J.K. Northcutt, N.A. Cox and M.A. Harrison, 2004. Identification of *Enterobacteriaceae* from washed and unwashed commercial shell eggs. J. Food Protect., 67: 2613-2616.
10. Komba, E.V.G., E.V. Komba, E.M. Mkupasi, A.O. Mbyuzi, S. Mshamu, A. Mzula and D. Luwumba, 2012. Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: Implications for public health. Tanzania J. Health Res., 14: 1-12.
11. Eisel, W.G., R.H. Linton and P.M. Muriana, 1997. A survey of microbial levels for incoming raw beef, environmental sources and ground beef in a red meat processing plant. Food Microbiol., 14: 273-282.
12. Kim, H.J., D. Kim, S.O. Song, Y.G. Goh and A. Jang, 2016. Microbiological status and guideline for raw chicken distributed in Korea. Korean J. Poult. Sci., 43: 235-242.
13. Bhandare, S.G., A.T. Sherikar, A.M. Paturkar, V.S. Waskar and R.J. Zende, 2007. A comparison of microbial contamination on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. Food Control, 18: 854-858.
14. Hoque, M.M., M.B. Inatsu, V.K. Juneja and S. Kawamoto, 2007. Antimicrobial activity of cloves and cinnamon extracts against food borne pathogens and spoilage bacteria and inactivation of *Listeria monocytogenes* in ground chicken meat with their essential oils. J. Food Sci. Technol., 72: 9-21.
15. Kaefer, C.M. and J.A. Milner, 2008. The role of herbs and spices in cancer prevention. J. Nutr. Biochem., 19: 347-361.

16. Bassole, I.H.N. and H.R. Juliani, 2012. Essential oils in combination and their antimicrobial properties. Molecules, 17: 3989-4006.
17. Tchombé, N.L., A. Louajri and M.H. Benajiba, 2012. Therapeutic effects of ginger (*Zingiber officinale*). ISESCO J. Sci. Technol., 8: 64-69.
18. Gephart, P., R.G.E. Murray, R.N. Costilow, E.W. Nester and W.A. Wood *et al*., 1981. Manual of Methods for General Bacteriology. In: Ueber Die Isolirte Färbung der Schizomyceten in Schnitt-und Trockenpräparaten, Fortschritte der Medcin, Gram. C. (Ed.)., ASM Press, Washington, D.C., USA., pp: 185-189.
19. Jordan, E.O., M.E. Caldwell and D. Reiter, 1934. Bacterial motility. J. Bacteriol., 27: 165-174.
20. Vaughn, R., N.B. Mitchell and M. Levine, 1939. The Voges-Proskauer and methyl red reactions in the Coli-aerogenes group. J. Am. Water Works Assoc., 31: 993-1001.
21. Difco, 1998. Difcomanula. 11th Edn., DifcoLaboratories, Detroit, MI., USA.
22. MacFaddin, J.F., 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd Edn., Williams and Wilkins, Lippincott, New York, ISBN: 0-683-05318-3.
23. Christensen, W.B., 1946. Urea decomposition as a means of differentiating *Proteus* and paracolon organisms from each other and from *Salmonella* and *Shigella* types. J. Bacteriol., 52: 461-466.
24. Eaton, A.D., L.S. Clesceri, E.W. Rice, A.E. Greenberg and M.A.H. Franson, 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edn., American Public Health Association, USA., ISBN-10: 9780875530475, Pages: 1200.
25. Harrigan, W.F. and M.F. McCance, 1976. Laboratory methods in food and Dairy Microbiology. 7th Edn., Churchill Livingstone, New York.
26. Sambrook, J. and D.W. Russell, 2006. Purification of nucleic acids by extraction with phenol: Chloroform. Cold Spring Harb. Protoc., 10.1101/pdb.prot4455.
27. Ramesh, P. and R.N. Okigbo, 2008. Effects of plants and medicinal plant combinations as anti-infectives. Afr. J. Pharm. Pharmacol., 2: 130-135.
28. Pelczar, Jr., M.J., R.D. Reid and E.C.S. Chan, 1977. Microbiology. 4th Edn., Tata McGraw-Hill, New Delhi.
29. Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
30. Valgas, C., S.M. de Souza, E.F.A. Smania and A. Smania Jr., 2007. Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol., 38: 369-380.
31. Jambalang, A.R., E.M. Buys and F.S. Botha, 2017. Bacterial species from retailed poultry eggs in Tshwane, South Africa: Implication for consumers. S. Afr. J. Sci., Vol. 113. 10.17159/sajs.2017/20160232.
32. Fayaz, S., G.A. Badroo, A. Ahmad, U. Rasool, R. Mustafa and M. Mudasir, 2017. Molecular characterization of enterotoxigenic *Bacillus cereus* species isolated from meat using conventional PCR and multiplex PCR. Int. J. Curr. Microbiol. Applied Sci., 6: 324-328.
33. Frazier, W. and D. Westhoff, 1988. Growth of Microorganisms at Low Temperature. In: Food Microbiology, Frazier, W.C. and D.C. Westhoff (Eds.)., 4th Edn., McGraw Hill, USA.
34. Puangpronpitag, D. and C. Sittiwet, 2009. Antimicrobial properties of *Cinnamomum verum* aqueous extract. Asain J. Biol. Sci., 2: 49-53.
35. Saeed, M., M. Nadeem, M.R. Khan, M.A. Shabbir, A. Shehzad and R.M. Amir, 2013. Antimicrobial activity of *Syzygium aromaticum* extracts against food spoilage bacteria. Afr. J. Microbiol. Res., 7: 4848-4856.
36. Roy, A.C., M.S. Islam, M.M. Haque, M.S. Hossain and M.A. Sayeed, 2015. Microbiological investigation of *Piper chaba hunter*. Int. J. Sci. Eng. Res., Vol. 6.
37. Yassen, D. and A.E. Ibrahim, 2016. Antibacterial activity of crude extracts of ginger (*Zingiber officinale* Roscoe) on *Escherichia coli* and *Staphylococcus aureus*: A study *in vitro*. Indo Am. J. Pharm. Res., 6: 5830-5835.
38. Gupta, C., A.P. Garg, R.C. Uniyal and A. Kumari, 2008. Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes. Afr. J. Microbiol. Res., 2: 247-251.