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FEASIBILITY AND EFFICACY STUDY OF SPICES IN MEAT PRESERVATION

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The systematic application of spices as natural food preservatives could be the key to withstanding different food-borne diseases and the frequent use of antibiotics could be reduced thereby. Eight indigenous spices were tested against six food-borne pathogens. The spice extracts were prepared by drying, grinding, and soaking into 95% ethanol and the antibacterial activity was evaluated by the well-diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the broth dilution method. The feasibility of spices as natural meat preservatives was then assessed through the application. The ethanol extracts of the spices potentially inhibited the test organisms. Maximum activity (zone of inhibition- ZOI) was recorded for garlic (17.83±2.48 mm) followed by black pepper (17±8.9 mm), black cumin (15.83±10.87 mm), and ginger (15.16±7.68 mm). For pathogens, the most susceptible was *B. cereus* (19.57±8.05 mm) followed by *Acinetobacter* sp. (18.14±1.34 mm), *E. coli* (16.28±1.88 mm), *S. aureus* (14.28±9.91 mm), *V. cholerae* (10.85±7.94 mm) and *Salmonella enterica* ser. Typhi (6.85±8.55 mm). Garlic exhibited the most effective and consistent inhibitory activity whereas black cumin exhibited the highest activity against *B. cereus* (34 mm). These results were highly comparable to the commercial antibiotics, e.g. Meropenem (28 mm). Against the *Salmonella* spp., ginger, cumin, and garlic demonstrated moderate inhibition (16 mm) whereas complete resistance was observed against other spices. The lowest MIC and MBC were recorded for black cumin against *B. cereus* (32 mg/ml and 64 mg/ml, respectively). But garlic was found to be the best candidate due to its lowest mean MIC (85.33±33 mg/ml), and MBC (170.66±66 mg/ml). Black cumin, garlic, and black pepper were efficient in reducing the total viable count of meat at 72 hours and hence could be developed as natural food preservatives.

Keywords: Spices, Antimicrobial potential, Meat Preservation, Efficacy

INTRODUCTION

Spices and herbs are a few daily agents in Bengali cuisine to enhance the taste and flavor of both cooked and raw foods. The use of these spices and herbs is in fact for traditional taste which people prefer even without knowing their health benefits. The history of their use as an enhancer of flavor and aroma in food, folk medicines and food preservatives among different cultures is common and long (1). With progression, the potential therapeutic values including antimicrobial, antioxidant and anticancer properties were discovered (2). With the advent of the new era of access to information, awareness and interest grew among people of all classes the spices and herbs due to food safety concerns with increased demand for systematic application in native foodstuffs as well as scientifically optimized combinations for maximum health benefits. Consequently, many natural food preservatives were derived from spices and herbs such as cuminaldehyde from cumin, eugenol from clove, cinnamaldehyde from cinnamon, etc. which are being used to preserve many processed foods nowadays (3).

The ability of spices, herbs, and other plant extracts to the preservation of foods is attributed to the presence of certain major bioactive compounds including phenolic acids, terpenes, aldehydes, flavonoids, and more which function as strong antioxidant and antimicrobial agents. Inhibition of microbial growth and lipid oxidation facilitates the extension of shelf-life, quality, and safety of food products (4,5). Hence, the wide and frequent application of these natural preservatives could aid in reducing chemical similitude and associated chronic deleterious health effects.

In Bangladesh, varieties of spices such as onion, garlic, ginger, bay leaves, turmeric, coriander, pepper, cumin, black cardamom, cinnamon, clove, black cumin, black pepper, black seed, etc. are used mainly in different combinations for cooking different dishes. A good number of spices were reported with antagonistic activities against several food-borne pathogens *in vitro* as well as in the prepared food (6–8). Food-borne outbreaks are common here in Bangladesh due to poor hygiene practices and a favorable climate for microbial growth. These outbreaks are noticed rarely only when severe illness erupts (9). Appropriate hygiene practices in personal life as well as in food preparation and the optimized application of spice could be the keys to

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reducing such cases in Bangladesh could be the keys to reducing such cases in Bangladesh. Hence, besides improving hygiene practices, potential spices for different food items should be screened following *in vitro* antimicrobial assay and microbial quality assessment of foods after application.

Spice-food combination depends mostly on traditional as well as personal tastes. The cooking of eat here involves a series of spices that extends the shelf life of the item along with the taste. On the of the contrary, storage of raw meat involves different techniques such as chilling/refrigeration, freezing, brining, curing, smoking, thermal processing, canning, dehydration, irradiation, chemicals, pressure processing, etc. so that the chances of pathogenic microbial growth, oxidation, andenzymatic spoilage is reduced (10–12). The use of spices in the preservation of raw meat at low temperatures was reported to be successful (13–15). Since the technique is a simple one,it could be used by the mass population for the safe storage of their meat. The objective of this study was to evaluate the antimicrobial potential of spice extracts against food-borne bacterial pathogens and their application in beef preservation.

MATERIALS AND METHODS

Bacterial Strains: Six pathogenic bacteria viz. *Acinetobacter* sp., *Bacillus cereus* (ATCC 14579), *Escherichia coli* (ATCC 25922), *Salmonella enterica* ser. Typhi (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *and Vibrio cholerae* (ATCC 14035) obtained from the Department of Microbiology, Primeasia University, Bangladesh, were stocked in a medium containing 20% glycerol in cryogenic vials at -70°C. Working cultures were maintained on Tryptic Soy Agar (TSA) slants at 4°C and fresh slants at specific time intervals were used for subculture.

Preparation of Ethanol Extracts from Spices: Seven common spices used in this study were black cumin (*Nigella sative*), black pepper (*Piper nigrum*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), cumin (*Cuminum cyminum*), mustard (*Brassica nigra*) and onion (*Allium cepa*). Fresh bulbs of garlic and onion, the fresh root of ginger, fresh dried fruits of black pepper, and the fresh seeds of cumin, mustard, and black cumin were cleaned, washed with sterile distilled water, sliced, and dried on trays in a hot air oven at 50°C the brittleness. The dried spices were then ground in a sanitized blender and 20.0 g of each spice powder was added aseptically into 80 ml of 95% ethanol and allowed to be soaked overnight at room temperature in a reciprocal shaking platform at 150 rpm (WIS-10, Wisecube, Germany). The separation of the ethanol fraction was done with a sterilized cheesecloth and filtered subsequently through a sterilized Whatman filter paper (No. 3). The ethanol fraction was then kept in an oven (Binder, USA) at 40°C to evaporate any residual ethanol. The extract was weighed and dissolved in ethanol to a concentration of 10 mg/ml (16), and the dried substance was kept in a sterile bottle under refrigerated condition for further use.

Inoculum preparation: The inocula of the test organisms were prepared by transferring one loop-full of the colony from TSA (Sigma, USA) medium into 9.0 ml of sterile Mueller-Hinton Broth (Difco, Sparks, MD) and incubated at 37°C for 5 to 6 hours. The bacterial cultures were compared with the McFarland turbidity standard (10^8 CFU/ml) prepared by mixing 0.05 ml of 1.175% Barium chloride dihydrate ($BaCl_2.2H_2O$), with 9.95 ml of 1% sulfuric acid (H_2SO_4) in a test tube with constant stirring (16, 17).

Agar well diffusion assay: The antibacterial activity of ethanol extracts of spices was evaluated following the agar well diffusion method (18). In brief, to prepare bacterial lawn of test pathogens, overnight liquid cultures in MHB medium at desired turbidity of 0.5 M McFarland standard were seeded on Muller Hinton Agar (MHA) plates with sterilized cotton swabs (16, 17). The cotton swabs were soaked in the liquid cultures and applied evenly in three directions at an angle of 60°C onto the surface of MHA plates. A sterile borer was then used to make 5 wells of 8.0 mm diameter in each plate, once the wet surface of the MHA medium was dried up. Ethanol extracts of the spices were poured separately in amounts of 100 µl into the wells along with negative control, the very ethanol used in the extraction process. After 12-15 minutes of diffusion time at room temperature, the plates were incubated at 37°C for 48 hours and the zone of inhibition (ZOI) was measured in 3 directions and recorded for the ethanol extracts. For a certain spice against pathogens, the diameter of the ZOIs more than 12 mm was considered as inhibition (19), diameters between 12-16 mm were considered moderately active, and greater than 16 mm were considered highly active. Each experiment was performed in triplicate and mean values were taken.

Determination of MIC and MBC: The MIC and MBC of the crude extracts were determined following the tube dilution techniques with MHB as diluents and growth medium (16). In brief, 1.0 ml of the reconstituted ethanol extract (512 µg/ ml) was added to 1.0 ml of MHB medium, and simulation of the fashion helped in obtaining a series of concentrations i.e. 512, 256, 128, 64, 32, 16 and 8 (µg/ml). Besides, the turbidity of the overnight grown test pathogens on MHB medium was adjusted to 0.5 M McFarland standard to be used as inoculums (16). Then, 100 µl of each inoculum was mixed well with the serially diluted crude spice extracts, and the vials retaining the mixtures were incubated at 37°C for 24 hours.

A vial devoid of any crude spice extract but pure diluent (1.0 ml) was kept as a negative control. The lowest concentrations of the extracts hardly demonstrating any visible growth were considered the MICs. One loop-full of the mixture from the MIC tubes was sub-cultured on TSA plates and incubated overnight at 37°C and the MICs yielding no growth were recorded as the MBCs.

Sample preparation: The beef sample was obtained from a local butcher shop in Dhaka and collected into insulated polystyrene boxes placed in ice and transported to the laboratory immediately using the standard microbiological procedure. The beef was washed with sterile saline in the bio-safety cabinet and cut into 50 g pieces with a sterile knife. The beef pieces were then soaked into the solutions of selected treatments i.e. 0.85% NaCl, vinegar, and separate solutions of extracts of all spices prepared in sterile distilled water. Raw beef pieces were also kept as no treatment. Beef pieces were then transferred into sterile containers and stored at room temperature for 3 days for further microbiological assessment. The experiment was performed in triplicates.

Microbiological analysis: The influence of the spice extracts on the microbiological quality of the beef was determined by estimating the Total Viable Count (TVC) of the treated beef samples after 72 hours of incubation at room temperature. In this connection, the beef pieces were transferred aseptically into separate stomacher bags containing 90 ml of sterile buffered peptone water solution and homogenized properly for 2 minutes. The beef homogenates were then diluted serially from 10^{-1}-10^{-8} folds in 0.85% NaCl solutions and 100 µl of each dilution was spread on the Nutrient agar plate. After overnight incubation at 37°C, all the plates were checked and the numbers of colonies were recorded. The numbers were then expressed as $\log_{10}$CFU/gm of beef.

RESULTS

Antibacterial activity of tested spices: The antibacterial assay revealed that the ethanol extracts of spices used in this study potentially inhibited the test organisms at varied levels (Table 1). Among them, *B. cereus* (19.57±8.05 mm) was the mostly inhibited bacterium followed by *Acinetobacter* sp. (18.14±1.34 mm), *E. coli* (16.28±1.88 mm), *S. aureus* (14.28±9.91 mm), *V. cholerae* (10.85±7.94 mm) and *Salmonella enterica* ser. Typhi (6.85±8.55 mm) by the spice extracts. Based on the inhibitory activity against the test organisms, the most active spice was garlic (17.83±2.48) followed by black pepper (17±8.9), black cumin (15.83±10.87), ginger (15.16±7.68), cumin (14±7.5), mustard (10.5±8.47) and onion (10±8.19). Garlic was most effective against *S. aureus* (22 mm) followed by *Acinetobacter* sp. (19 mm), *E. coli* (18 mm), *B. cereus* (17 mm), *S. enterica* ser. Typhi (16 mm), and *V. cholerae* (15 mm). Garlic also demonstrated the most consistent inhibitory activity (sd-±2.48) against all organisms although the black cumin exhibited the highest activity (ZOI= 34 mm) against *B. cereus* whereas garlic was moderately active. The inhibitory level of black pepper was very close to garlic. Nevertheless, black pepper was ineffective against *S. enterica* ser. Typhi (no inhibition at all), caused maximum inhibition to *E. coli* (18 mm) and *V. cholerae* (21 mm). *S. aureus* and *Acinetobacter* sp. were also moderately inhibited by Black pepper. Black cumin, ginger, and cumin inhibited the organism at a moderate level. Interestingly, *S. enterica* ser. Typhi was inhibited equally (16 mm) by ginger, cumin, and garlic whereas black cumin, black pepper, mustard, and onion were not at all inhibiting. Cumin and garlic inhibited *S. aureus* maximum (18 mm) but onion and mustard did not

exhibit any inhibitory activity. Onion inhibited *E. coli* maximum (18 mm) besides black pepper and garlic. All of the antibiotics used in this study as controls demonstrated strong bacteriocidal activity except AP10, NA30, and NI300. *Acinetobacter* sp. was found to be completely resistant against AP10 and NA30 and *V. cholerae* was completely resistant against NA30 and NI300. The average diameters of zones of inhibition against the test organisms by AP10 as well as NI300 were around 15 mm and by NA30, it was 11.16±8.9. Again, *Acinetobacter* sp. and *V. cholerae* demonstrated resistance against these antibiotics to some extent (Table 1).

Table 1: Antibacterial potentials of tested spices and commercial antibiotics.

Agents	***B. cereus***	***E. coli***	***Acinetobacter***	***S. aureus***	***V. cholerae***	***Salmonella***	**Mean±SD**
Black Cumin	34	13	16	17	15	0	15.83±10.87
Black pepper	26	18	17	20	21	0	17±8.9
Ginger	21	16	19	19	0	16	15.16±7.68
Onion	12	18	18	0	12	0	10±8.19
Mustard	14	16	20	0	13	0	10.5±8.47
Cumin	13	15	18	22	0	16	14±7.5
Garlic	17	18	19	22	15	16	17.83±2.48
Mean±SD	19.57±8.05	16.28±1.88	18.14±1.34	14.28±9.91	10.85±7.94	6.85±8.55	
GEN10	24	22	20	22	23	14	20.83±3.6
Lev 5	22	26	16	22	25	20	21.83±3.6
AP 10	15	21	0	18	16	20	15±7.6
IPM 10	26	21	18	22	10	25	20.33±5.8
CIP 5	23	27	17	22	22	15	21±4.3
NA 30	13	20	0	16	0	18	11.16±8.9
MEM 10	30	25	22	28	22	18	24.16±4.4
NI 300	24	20	18	18	0	14	15.66±8.33
Mean±SD	22.12±5.59	22.75±2.81	13.87±8.75	21±3.7	14.75±10.26	18±3.74	

Note: Gentamicin (GEN 10µg), Levofloxacin (LEV 5µg), Ampicillin (AP 10µg), Imipenem (IPM 10µg), Ciprofloxacin (CIP 5µg), Nalidixic acid (NA 30µg), Meropenem (MEM 10µg), Nitrofurantoin (NI 300µg).

MIC and MBC of spice extracts: The sensitive bacteria, as revealed through the antibacterial assay of ethanol extract of spices, were further exposed to different dosages comprised of gradient concentrations for the determination of MIC and MBC. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the spices against the test organisms were determined thus. It was found that the MIC and MBC of ethanol extracts of spices against *B. cereus* were in the range of (32-256 mg/ml) and (64-512 mg/ml) respectively. In both cases, black cumin was found to be the most efficient with a minimum of 32 mg/ml (MIC) and 64 mg/ml (MBC) whereas black pepper, ginger, and garlic exerted the same at a slightly higher dose (MIC- 64 mg/ml and MBC- 128 mg/ml) (Table 2). For *E. coli*, MIC and MBC were in the range of (64-256 mg/ml) and (128-512 mg/ml), respectively and the lowest concentrations were recorded for garlic (MIC- 64 mg/ml and MBC- 128 mg/ml). For *Acinetobacter sp.*, MIC and MBC were in the range of (64-128 mg/ml) and (128-256 mg/ml) respectively and the inhibitory dosages were almost the same for black pepper, onion, mustard, garlic, and cumin i.e. MIC- 64 mg/ml and MBC- 128 mg/ml. For *S. aureus*, MIC and MBC were in the range of (64-128 mg/ml) and (128-256 mg/ml) respectively and the lowest dosages were observed for garlic and cumin (MIC- 64 mg/ml and MBC- 128 mg/ml). For *V. cholerae*, MIC and MBC were in the range of (64-256 mg/ml) and (128-512 mg/ml) respectively and the lowest dose was recorded for black pepper. For *Salmonella* ser. Typhi, MIC and MBC were in the range of (128-256 mg/ml) and (256-512 mg/ml), respectively with garlic producing the most inhibitory effect (MIC- 128 mg/ml and MBC- 256 mg/ml). The spices, earlier proven to be inefficient, were not considered in this case and hence remarked with asterisks (*: not performed).

Table 2: MIC and MBC of extracts (mg/ml) of all spices against foodborne bacterial pathogens.

Organisms	**Black cumin**		**Black pepper**		**Ginger**		**Onion**		**Mustard**		**Garlic**		**Cumin**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B. cereus	32	64	64	128	64	128	256	512	256	512	64	128	256	512
E. coli	256	512	128	256	256	256	128	256	128	256	64	128	256	512
Acinetobacter sp	128	256	64	128	128	256	64	128	64	128	64	128	64	128
S. aureus	128	256	128	256	128	256	*	*	*	*	64	128	64	128
V. cholerae	128	256	64	128	*	*	256	512	256	512	128	256	*	*
S. enterica	*	*	*	*	256	256	*	*	*	*	128	256	256	512

Note: * =not performed.

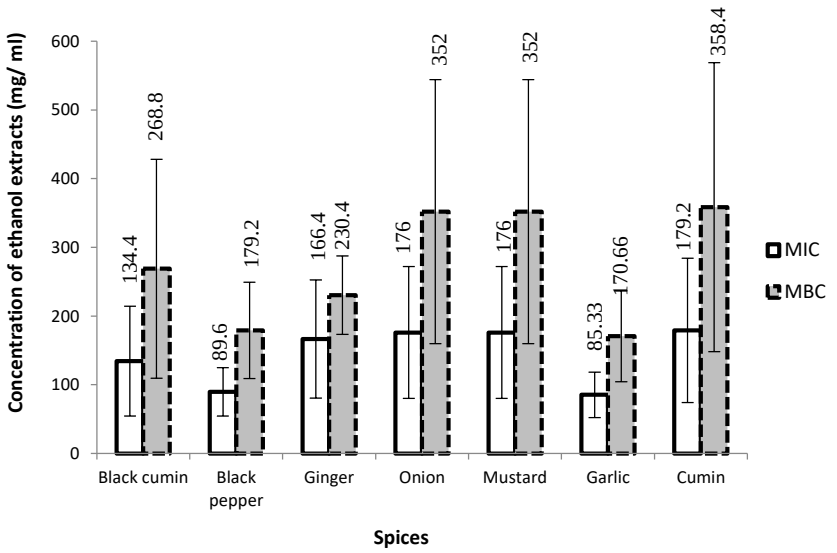

Figure 1: Average MIC and MBC of ethanol extracts of spices against the tested food-borne pathogens. While considering the overall inhibitory effects of ethanol extracts of spices against the test organisms, garlic was found to be the best candidate due to its lowest average MIC (85.33±33 mg/ml) and MBC (170.66±66 mg/ml) and inhibited all the pathogens consistently. Black pepper (89.6±35 mg/ml) was very close to garlic and black cumin was also found to produce higher inhibitory effects against the test organisms. But black pepper and black cumin failed in inhibiting *S. enterica* ser. Typhi.

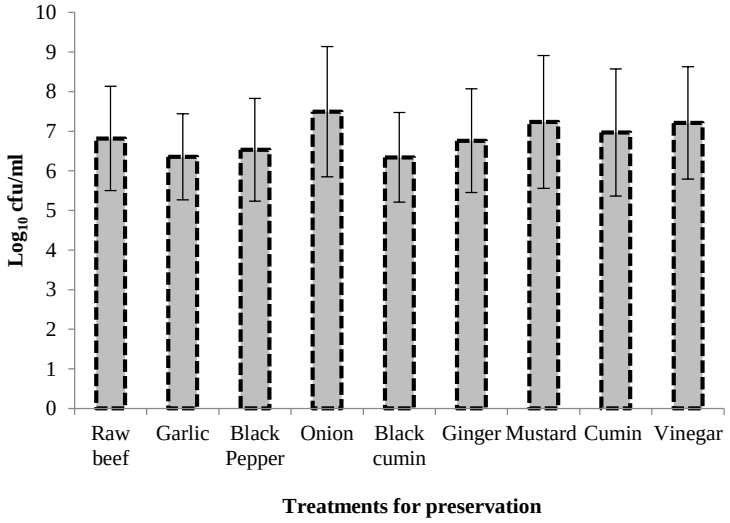

Figure 2: Effect of spice extracts on the microbial load of raw beef after 72 hours of treatment. Treating raw meat with spice extracts, especially garlic, black pepper, black cumin and ginger was found to inhibit microbial growth.

The efficiency of spice extracts in beef preservation: The total viable count (TVC) of raw beef, as estimated before any treatment, was 6.55×10^6 cfu/ml. After 72 hours of incubation at 37°C, the microbiological quality of both treated and raw meat was compared. It was revealed that the spice extracts exerted both bacteriocidal and bacteriostatic effects (Fig. 2). Black cumin, garlic, black pepper, and ginger caused a 66.66%, 65.55%, 48.18%, and 11.93% reduction in the total viable count of the treated beef respectively whereas the microbial growth was enhanced in the untreated raw beef as revealed from the TVC which was too numerous to count (TNTC). Although cumin did not reduce the TVC, it hampered the normal multiplication of the microorganisms, thus causing bacteriostatic effects. On the other hand, onion, mustard, and vinegar retarded the growth rate of the microorganisms.

DISCUSSION

Food could be contaminated with pathogenic microorganisms through a series of natural events and artifacts in its journey from farm to fork. The

challenges with both pathogenic and non-pathogenic microbes are being augmented worldwide with times since multidrug resistance and the transfer of these antibiotic resistance genes are reaching beyond control. Uncontrolled, unnecessary, and sometimes unprescribed use of antibiotics is causing such damage which should be recovered in a holistic approach. Both reducing unnecessary use of antibiotics and ensuring safe foods devoid of pathogenic microbes should be implemented simultaneously besides other practices. The access of pathogens could be kept limited by sanitizing fruits and vegetables before consumption or the microbial load must be maintained below the thresholds for long-term storage. The latter is usually achieved with chemical preservatives which very often cause chronic health complexities and their substitution is highly recommended. The study was, therefore, aimed at the assessment of the antimicrobial potentials of indigenous spices and their feasibility as natural food preservatives.

The common kitchen spices were selected in this study and their ethanol extracts were tested against 6 important food-borne pathogenic bacteria. Since the ethanol fraction of herbs and spices was reported to contain potential anti-microbial compounds such as terpenes and terpenoids (16, 20), this very fraction of spices was prepared and tested. It was observed from the study that the ethanol extracts of the spices potentially inhibited the test organisms at a varied level. The test organisms were comprised of 2 gram-positive bacteria (*B. cereus* and *S. aureus*) and 4 gram-negative bacteria (*Acinetobacter* sp., *E. coli*, *S. enterica* ser. Typhi, and *V. cholerae*). It is well known that spices are more active against gram-positive bacteria than gram-negative ones (7, 21). Here in this study, although *B. cereus* (19.57±8.05 mm) was the mostly inhibited bacterium, *Acinetobacter* sp. (18.14±1.34 mm), *E. coli* (16.28±1.88 mm) were more susceptible to the spices than gram-positive *S. aureus* (14.28±9.91 mm). It could therefore be an interesting finding since spices were exerting inhibitory activity against gram-negative bacteria. More interestingly, the average bioactivity of spices against *Acinetobacter* sp. was 18.14±1.34 mm (ZOI) whereas 23.5% lower efficacy (13.87±8.75) was observed for the commercial antibiotics. Again, the spices were more consistent in inhibiting the *Acinetobacter* sp. than the commercial antibiotics. This finding is in accordance with the report of Rath et al. where the *Acinetobacter baumannii* was found to be resistant against almost all tested antibiotics but sensitive against the methanolic extracts of spices (21.81±3.56) (22). On the contrary, *S. enterica* ser. Typhi (6.85±8.55) and *Vibrio cholerae* (10.85±7.94) were found to be less inhibited than other 4 test organisms by the spice extracts. Their sensitivity against the antibiotics was also moderate.

While compared to commercial antibiotics, the tested spices extracts showed convincing antibacterial potentials (Table 1). Meropenem (MEM 10µg), Imipenem (IPM 10µg), Ciprofloxacin (CIP 5µg), Gentamicin (GEN 10µg), and Levofloxacin (Lev 5µg) (Oxoid, UK) were found to be more active than the spices but not exceeding 35.5%. Meropenem (MEM 10) demonstrated maximum activity (35.5% more than that of garlic) followed by Lev 5 (22.4% more), CIP 5 (17.78% more), GEN10 (16.8% more), IPM 10 (14% more). Since black cumin demonstrated maximum antibacterial activity (34 mm) against *B. cereus*, even more than that of MEM (30 mm), this spice could be the source of a very efficient and safe food preservative. This resembled other study reports that Black cumin strongly inhibited *B. cereus* (6, 23). Apprehending cases were the complete resistance of *Acinetobacter* sp. against Ampicillin (AP10) and Nalidixic acid (NA30), and *V. cholerae* against NA30 and Nitrofurantoin (NI 300). Fortunately, *Acinetobacter* sp. was inhibited by all spices which suggest that spices could be, besides their uses as food preservatives, the sources of efficient antimicrobial drugs, and *V. cholerae* was inhibited most by black pepper followed by black cumin and garlic. Hence, for instance, Black cumin highly active against *B. cereus* (ZOI: 34 mm), should be analyzed more to develop efficient and safe food preservatives to ward off bacillary dysentery. Garlic extract demonstrated satisfactory inhibition (17.83±2.48) against all tested organisms even *S. aureus*, *V. cholerae*, *S. ser*. Typhi which suggests that garlic should be a must spice in the dish or as a salad dressing item and effective drugs could be developed from it.

Then, MIC and MBC of those ethanol extracts were estimated. Garlic demonstrated a consistent inhibition of all test organisms at a low concentration (mean value: 85.33 mg/ml) followed by black pepper (mean value: 89.6 mg/ml). Among the spices, only garlic was pepper which were 6.35, 6.34, and 6.53 log CFU/gm (Fig. 2), respectively. On the other hand, four spice extracts (cumin, mustard, ginger, and onion) treatments resulted in a slow increase of TVC samples after 72 hours. The vinegar treatment resulted in higher TVC in the beef after 72 hours (Fig. 3). The spices, especially garlic, black pepper, and black cumin were found to possess satisfactory levels of broad-spectrum antimicrobial activities and could be utilized as safe and effective natural food preservatives.

CONCLUSION

The efficacy of the spices, especially garlic, black pepper, and black cumin, in inhibiting food-borne pathogens as well as in beef preservation could be observed in this study. Since few antibiotics were found to be inefficient against certain pathogens whereas spice extracts inhibited them successfully, more attention should be on the systematic use of spices in food preparation rather than the frequent use of antibiotics.

CONSENT AND ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors declare that there is no competing interest.

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