Time-Kill Kinetics of Lipid Fractions Isolated from Condiments against Foodborne Pathogens

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SUMMARY

Lipid fractions that are extracted from condiments have a wide array of biological potential and are commonly utilized for medicinal and culinary applications. This investigation aims at determining the antimicrobial potential of lipid fractions isolated using two different solvent systems against five foodborne pathogens. The antibacterial efficacy was tested after 0, 1, 2, 3 and 24 h of incubation with the active agent. The leakage of cellular content was assessed at 1 and 2 h of incubation. Scanning electron microscope (SEM) images were obtained after 18 h of contact time with lipid fractions at their minimum inhibitory concentration (MIC). From the results obtained from time-kill and cell constituents release tests, it could be concluded that during 3 and 1 h of incubation, the lipid fractions were more potent against Gram-negative isolates (*Escherichia coli* ATCC 8739). However, prolonged incubation with the active agent inhibited Gram-positive isolate, *i.e. Listeria monocytogenes* ATCC 13932. SEM images of treated microorganisms also confirmed the inhibitory action of selected lipid fractions against all the tested pathogens. The cellular morphology of the bacteria was completely altered after 18 h of incubation with the lipid fractions. The results of the present study corroborate significant inhibitory effects and disruption in bacterial cell integrity following prolonged incubation with these lipid fractions. The results also affirm the use of the tested lipid fractions in food systems.

Key words: time-kill kinetics, scanning electron microscopy, cell constituent release, foodborne pathogens

INTRODUCTION

Pathogens are ubiquitous and are associated with every phase of human life. Bacteria with a potential to cause severe ailments are found in soil, water bodies or even in our food items and may cause life-threatening illnesses in humans. Therefore, to prevent any such instances recent techniques in the field of food technology and microbiology have been applied to ensure consumer safety and a safer food supply. In the past few decades, the cases of foodborne infections have increased dramatically due to utilization of undercooked and minimally processed ready-to-eat products, including hot dogs, salami, sausages, dairy products, primarily ice cream, or fruits and vegetables (*1*). Propagation of bacteria during low temperature storage, contamination by pathogens and their resistance to disinfection are alarming issues regarding minimally processed products, since these items are utilized without any prior treatment or processing (*2*). Grazing animal and poultry meat may be a source of foodborne pathogens. Feed supplements that are basically animal byproducts may also act as a carrier to transfer microorganisms to other animals. On the other hand, fertilization of fruits and vegetables with untreated manure can also contaminate soil or water supplies with pathogens. The contaminated water supply may also transmit bacteria or food viruses, which include *Listeria*, *Salmonella*, *Clostridium*, *Yersinia*, *Campylobacter*, *Shigella* and hepatitis A. These pathogens may be present in low numbers or even in low infectious doses (10–1000 bacterial cells). Due to increasing awareness of food safety issues such as foodborne ailments and their implications, consumers have become much more aware of

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the health concerning problems. Disinfection using artificially synthesized antimicrobial agents is often utilized for minimally processed foods (MPF) to protect the product, but these compounds possess some adverse effects and have limitations in their usage, such as low antibacterial potency and may result in the development of cancers (3). The inability of these chemicals to eliminate microorganisms from fruits and vegetables may be due to plethora of factors: hydrophobicity of the waxy cuticle of vegetables and fruits, which prevents the disinfectant from reaching the surface; the formation of biofilms that can protect the microorganism from the lytic effects of such agents; and inactivation of these disinfectants when they come into contact with the host tissues. Therefore, to achieve food safety, there is an elevated consumer demand for substituting artificially synthesized antibacterial agents with natural alternatives (4). The benefit of natural antimicrobial agents is that they have a wide spectrum of application without toxic effects on humans, improved sensory attributes and the ability to extend storage life of MPF (5). Earlier studies have indicated that bay leaf (*Laurus nobilis*), black cumin (*Nigella sativa*) seeds, fennel (*Foeniculum vulgare* Mill.) seeds and coriander (*Coriandrum sativum*) seeds possess antibacterial activity against pathogenic and food spoilage pathogens (6-9). *In vitro* studies have elaborated that lipid fractions have promising antibacterial potential. However, when these lipid fractions are applied to food matrices directly, relatively larger quantity of these active agents is required, making them organoleptically unacceptable (10). To avoid such instances, these lipid fractions need to be nanosized or encapsulated to balance antimicrobial potency and sensory acceptability (11). The objectives of this study are to report the time-kill kinetics of lipid fractions against five food isolates, *i.e. Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 13932, *Vibrio parahaemolyticus* ATCC 17802, *Bacillus cereus* ATCC 11778 and *Vibrio alginolyticus* ATCC 17749 when treated with lipid fractions obtained from two different solvent systems. Cell constituent release measured spectrophotometrically at 260 nm and scanning electron microscopy of treated and untreated (control) bacterial isolates were also compared to evaluate the potency of these antibacterial agents for application in food matrices without any possible hazards.

MATERIALS AND METHODS

Materials

Sodium chloride, phosphate-buffered saline, tryptone soy broth (TSB), Müller-Hinton agar (MHA) and broth were purchased from Thermo Fisher Scientific Oxoid Ltd. (Basingstoke, Hampshire, UK) and dimethyl sulfoxide from Fisher BioReagents (Roskilde, Denmark) were used in this study.

Extraction of lipid fractions

The dried herbs were collected from a local grocery store in Karachi, Pakistan (25.0700°N, 67.2848°E) and the tested lipid fractions were obtained using the solvent extraction system

according to the method of Cheikh-Rouhou *et al.* (12). The main aim was to evaluate the antibacterial potential of culinary herbs including bay leaf (Lauraceae family), black cumin (Ranunculaceae family), fennel seeds and coriander seeds (Umbellifer family) against foodborne pathogens. These herbs were selected due to the ease of their availability and common use as food ingredients. The lipid fractions used in this study were methanolic and ethanolic lipid fractions of fennel seeds, black cumin seeds, bay leaf and coriander seeds. The lipid fractions were extracted fresh to preserve the antibacterial potency and stored at freezing temperature until analyzed.

Concentration of lipid fractions used in time-kill assay

The selected lipid fractions did not yield results against all the tested microorganisms reported by Naeem *et al.* (13). Only those lipid fractions that gave antibacterial activity against the tested pathogens were selected for this research. The MICs of selected lipid fractions against food pathogens are shown in **Table 1** (13).

Bacterial cultures

Test microorganisms were foodborne pathogens, *i.e. Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 13932, *Vibrio parahaemolyticus* ATCC 17802, *Bacillus cereus* ATCC 11778 and *Vibrio alginolyticus* ATCC 17749, and were procured from Food Safety Research Laboratory, Department of Microbiology, University of Karachi, Pakistan. Each assay was carried out in three batches.

Time-kill studies

In vitro determination of antibacterial activity of lipid fractions included total viable count of the tested pathogens after the supplementation of the lipid fractions at minimum inhibitory concentration (MIC) according to the method of Hsouna *et al.* (14). For time-kill assay, test tubes containing bacterial cultures (approx. 10^8 CFU/mL) were inoculated with eight different lipid fractions at their MICs against respective bacteria. The concentrations of lipid fractions ranged from 62.5 to 1000 µg/mL. The dilutions of the used lipid fractions were prepared in 40 % dimethyl sulfoxide (DMSO). The volume was made up to 5 mL using physiological saline solution (0.8 %). After contact time of 0, 1, 2, 3 and 24 h, 100 µL of the sample were transferred to 900 µL of sterile saline solution. Subsequently, 0.1 mL of this diluted sample was spread on the surface of solidified Müller-Hinton agar plates. The control and test plates were incubated at 37 °C and bacterial counts were enumerated. The negative control, *i.e.* 40 % DMSO, was also run using the same protocol.

Cell constituent release

The release of intracellular components was measured spectrophotometrically at 260 nm by the method of Di Pasqua *et al.* (15). Working cultures of bacterial isolates were ob-

Table 1. Minimum inhibitory concentration (MIC) of lipid fractions against foodborne pathogens

Food pathogen tested	γ(MIC)/(μg/mL)							
	MN	EN	MF	EF	MC	EC	MB	EB
Escherichia coli ATCC 8739	500	250	N/D	N/D	250	1000	250	250
Listeria monocytogenes ATCC 13932	250	1000	1000	250	250	1000	250	500
Vibrio parahaemolyticus ATCC 17802	N/D	250	N/D	N/D	N/D	1000	250	N/D
Vibrio alginolyticus ATCC 17749	250	250	N/D	500	N/D	1000	N/D	250
Bacillus cereus ATCC 11778	250	250	1000	N/D	N/D	500	250	1000

N/D=No detection of antimicrobial activity hence no MIC. MN, MF, MC, MB, EN, EF, EC and EB stand for methanolic and ethanolic lipid fractions of black cumin, fennel seeds, coriander seeds and bay leaf, respectively. Reproduced from Naeem *et al.* (*13*)

tained. After centrifugation (Multifuge™ 1S centrifuge; Heraeus, Neston, Cheshire, UK) at 3000×*g* for 15 min, the cells were washed thrice and transferred to phosphate-buffered saline. The cell suspension (100 mL) was added to lipid fractions at their MICs and incubated in an orbital shaker (ES-20; CBM-Scientific, West Lothian, Scotland, UK) maintained at 150 rpm and (35±2) °C. A control was also prepared using the same method but with the absence of lipid fractions. After 1 and 2 h of incubation period, 10 mL of the sample were filter-sterilized through 0.2-μM pore size Corning (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) membrane filter. The effect of lipid fraction on the integrity of cell wall was determined by measuring the absorbance of the filtrate at 260 nm using UV-Vis spectrophotometer (1240 Mini; Shimadzu, Tokyo, Japan). The test tubes with tested bacteria without lipid fractions were used as a control. The blank control was sterile physiological saline.

Scanning electron microscopy

The morphological changes in selected bacteria treated with lipid fractions were determined using scanning electron microscopy (SEM) analysis. Selected foodborne pathogens, namely *Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 13932, *Vibrio parahaemolyticus* ATCC 17802, *Bacillus cereus* ATCC 11778 and *Vibrio alginolyticus* ATCC 17749, were incubated in tryptone soya broth at 37 °C for 18 h. The bacterial count was 1.5·10⁸ CFU/mL. Lipid fractions were supplemented at their ½ MICs to centrifuge tubes and labelled as treated. Control tubes having bacterial suspensions were also run alongside. All suspensions were incubated at 37 °C for 18 h. The cells were dehydrated using 70, 80 and 90 % ethanol for 10 min each. After dehydration, the specimens were coated with gold in an ion coater up to 300 Å using smart coater with an ion sputtering device (model JFC-1500) and energy-dispersive X-ray spectroscopy detector (model EX-54175JMU; JEOL, Tokyo, Japan). Finally, the disruption in bacterial cell morphology was observed with a SEM (JSM-6380A; JEOL) at Centralized Science Laboratories, University of Karachi, Pakistan.

Statistical analysis

Analysis of variance (ANOVA) was used to calculate significant differences between the mean values. Duncan's test (p≤0.05) was used to separate the mean values by employing SPSS software v. 23 (*16*).

RESULTS AND DISCUSSION

Bactericidal mechanism

The time-kill assay of MIC of respective lipid fraction against food isolates was performed (**Fig. 1-Fig. 5**). Generally, all the ethanolic lipid fractions had MIC of 62.5 μg/mL, which is the lowest concentration ever reported to be tested against selected foodborne pathogens. However, when these MICs were tested through agar well diffusion assay, the same concentration of lipid fractions failed to generate any such results. The difficulty in diffusion through the agar matrix may be the possible reason for not producing positive results. Ethanolic lipid fractions of bay leaf and black cumin seeds inhibited all the tested food pathogens, indicating that they are highly rich in antimicrobial components. Lipid fractions of black cumin and bay leaf were successful in inhibiting a wide range of pathogens when tested *in vitro*. The data of time-kill assay indicate that the lowest log CFU/mL was observed of *Listeria monocytogenes* ATCC 13932 when treated with methanolic extracts of lipid fraction of coriander seeds at 1000 μg/mL after 24 h of incubation. The reason behind this activity may be the chemical composition of the outer cell membrane of Gram-negative bacteria. It provides the hydrophilic attribute to the bacterial surface, thereby acting as a potent perme-
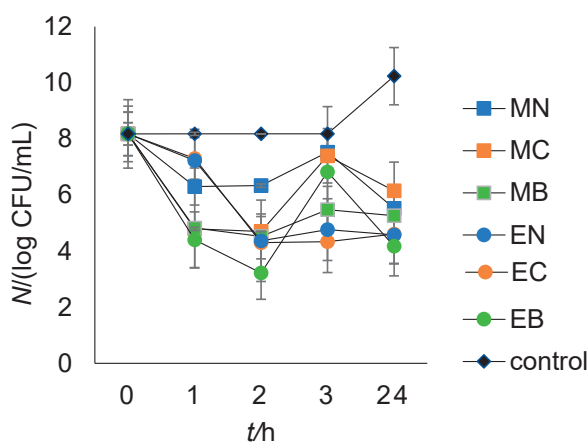

Fig. 1. Effect of different lipid fractions on the viability of *Escherichia coli* ATCC 8739. The vertical bars represent the standard error of mean values of three replicates. MN, MC, MB, EN, EC and EB stand for methanolic and ethanolic extracts of lipid fractions of black cumin, coriander seeds and bay leaf, respectively, at their minimum inhibitory concentrations (MICs)
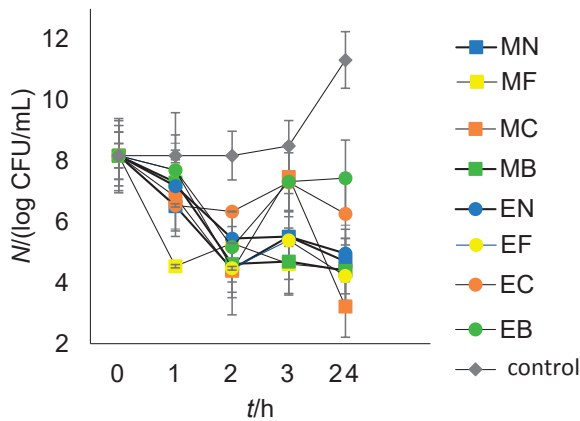
Fig. 2. Effect of different lipid fractions on the viability of *Listeria monocytogenes* ATCC 13932. The vertical bars represent the standard error of mean values of three replicates. MN, MF, MC, MB, EN, EF, EC and EB stand for methanolic and ethanolic extracts of lipid fractions of black cumin, fennel seeds, coriander seeds and bay leaf, respectively, at their minimum inhibitory concentrations (MICs)
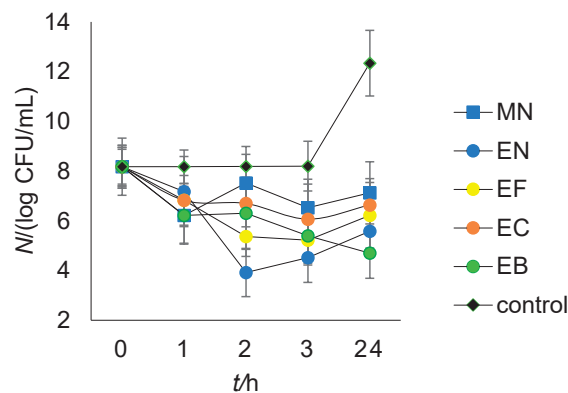

Fig. 4. Effect of different lipid fractions on the viability of *Vibrio parahaemolyticus* ATCC 17802. The vertical bars represent the standard error of mean values of three replicates. MB, EN and EC stand for methanolic extract of lipid fraction of bay leaf, and ethanolic extracts of lipid fractions of black cumin and coriander seeds, respectively, at their minimum inhibitory concentrations (MICs)

Fig. 3. Effect of different lipid fractions on the viability of *Vibrio alginolyticus* ATCC 17749. The vertical bars represent the standard error of mean values of three replicates. MN, EN, EF, EC and EB stand for methanolic extract of lipid fraction of black cumin and ethanolic extracts of lipid fractions of black cumin, fennel seeds, coriander seeds and bay leaf, respectively, at their minimum inhibitory concentrations (MICs)

Fig. 5. Effect of different lipid fractions on the viability of *Bacillus cereus* ATCC 11778. The vertical bars represent the standard error of mean values of three replicates. MN, MF, MB, EN, EC and EB stand for methanolic extracts of lipid fractions of black cumin, fennel seeds and bay leaf, and ethanolic extracts of lipid fractions of black cumin, coriander seeds and bay leaf, respectively, at their minimum inhibitory concentrations (MICs)

ability hurdle to different molecules (*10*). As Gram-positive bacteria lack this outer envelope, the hydrophobic components of lipid fractions had a direct interaction with the phospholipid bilayer of the cellular envelope generating their action, resulting either in elevated ion exchange and leakage of important intracellular components, or in dysfunction of enzymatic pathways in bacterial cells (*17*). However, after 3 h of incubation, the lowest bacterial count was observed of *E. coli* ATCC 8739 when tested against ethanolic lipid fraction of coriander. Time-kill kinetic studies showed that all the treated food isolates had similar trend of inhibition, *i.e.* maximum inhibition was observed after 2 h of incubation when lipid fractions were supplemented in the media. After 3 h, rise in bacterial count was observed followed by drop in the cell
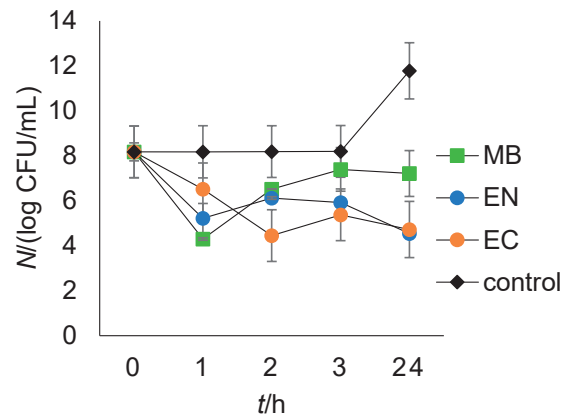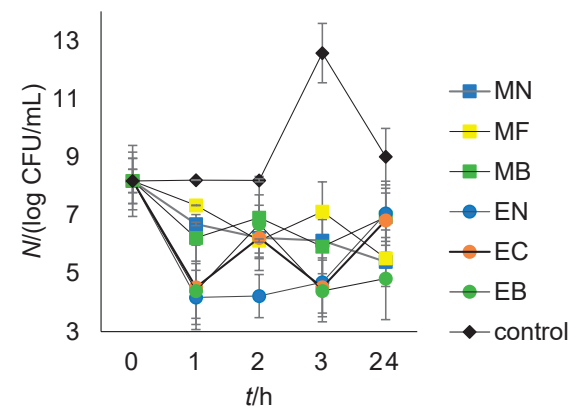
numbers after prolonged 24 h of incubation. However, bacterial counts increased in the control tubes. It could be concluded from this observation that the inhibitory phase of these lipid fractions was within 2 h, but after 24 h of incubation, the cells were inhibited due to extended contact with these lipid fractions. The most potent lipid fraction found from the results obtained from TVC after 24 h of incubation was that of bay leaf against nearly all the tested pathogens. When comparing the results of methanolic and ethanolic lipid fractions, it can also be concluded that the ethanolic lipid fractions were more potent in reducing the bacterial counts of all the tested food pathogens. The reason behind this activity is the ability of ethanol solvent to extract a large number of antioxidants, total phenolic and total flavonoid compounds. Due to higher concentration of

these compounds, which are the main contributors to antibacterial potential, higher antimicrobial activity is demonstrated by ethanolic lipid fractions (*18*). It is a well acknowledged fact that higher concentration of lipid fraction is needed to inhibit bacteria in food matrices than in laboratory media. Due to alterations in the organoleptic attributes of food, it would not be feasible to supplement a sufficient concentration of lipid fraction to have bactericidal effect. However, lower concentrations that lead to bacteriostatic conditions are enough for the food safety if the contaminating bacteria are low in numbers. The results reported in this study indicated that these lipid fractions are inhibitory at low concentration of 62.5 μg/mL, which would not interfere with the organoleptic attributes if incorporated in the packaging material of minimally processed foods. *Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 13932, *Vibrio parahaemolyticus* ATCC 17802, *Bacillus cereus* ATCC 11778 and *Vibrio alginolyticus* ATCC 17749 are the most important pathogens of great concern to food industry. *Vibrio alginolyticus* is present in seafood (*19*) and may cause gastrointestinal tract disorders upon ingestion of improperly cooked food items. *E. coli* and *L. monocytogenes* are commonly present in water and food items that are contaminated and did not receive any processing prior their consumption (*20*), whereas *B. cereus* may be present in rice and leftovers which are not stored at temperatures optimal for shelf life (*21*). As all these pathogens can cause life-threatening diseases, it is necessary to identify doses of natural antimicrobial agents to combat them without any toxic side effects.

Cellular constituent leakage

In this study, a significant increase in the cellular constituent leakage was observed when treating the tested bacteria with lipid fractions at their MICs compared to control (untreated). After 120 min of incubation with different lipid fractions, increase in the absorbance of the filtrates obtained from all the tested bacterial cells was observed (**Table 2**). When Student's *t*-test was applied to compare the activity of methanolic and ethanolic lipid fractions, data were found to be statistically significant. This indicates that there were no differences between both groups. This increase was lower in control samples. Therefore, it is concluded that the antibacterial mechanism of lipid fractions starts with the disruption of the cellular envelopes, followed by loss in intracellular constituents, which ultimately caused cell death. At 60 min of incubation, leakage of intracellular components was the highest in *E. coli* ATCC 8739 when it was treated with ethanolic lipid fraction of black cumin. However, the highest constituent release after 120 min of contact time was observed with *Listeria monocytogenes* ATCC 13932 when treated with lipid fraction of bay leaf. These observations showed that the longer the time of contact of bacterial cells with lipid fractions, the higher the inhibitory effects observed on bacterial cells with subsequent cell lysis.

The cellular constituents that are leaked and are detected at 260 nm from the filtered residues of food isolates can be utilized to assess the antibacterial potential of lipid fractions against foodborne pathogens (*22*). In this study, increase in

absorbance of the treated samples showed that the release of cell constituents into the broth increased due to cell wall disruption of tested pathogens by lipid fractions. The leakage of intracellular materials may elevate the cell wall permeability. The intracellular ingredients that were lost comprised mainly Na^+, K^+ and Ca^{2+}. However, in more intense cases, reducing sugars and cellular biomolecules such as nucleic acids and proteins are also lost during this transport. The leakage of these vital components may result in the irreversible destruction of the cellular morphology, which was further confirmed by SEM. The ions including Na^+, Ca^{2+} and K^+ are vital for maintaining the adenosine triphosphate (ATP) pool and metabolic regulation, so the transport of solutes can alter the structure of cellular membrane and may have a detrimental effect on the metabolic pathways in cell culminating in cell lysis (*23*). The molecules that constitute the living components of the cell include nucleic acids and amino acids and are present intracellularly throughout the cell cytoplasm. These molecules play a vital role in the process of translation, transcription and DNA replication, and if these moieties are disrupted, it may have a negative impact on cell physiology (*24*).

Changes in cellular morphology

The changes in cell membrane morphology were determined using SEM. The morphology of bacterial cells was completely altered and was observed only in treated cells as compared to untreated/control cells (**Fig. 6**). Different researchers have reported the effect of different antibacterial essences on tested bacterial isolates (*25-28*). The untreated cell membranes of all tested microorganisms had even and smooth surfaces. However, destructive changes were observed on the cellular membrane morphology of treated cells. Cells exposed to lipid fractions for 18 h showed deformities in the structure such as appearance of orifices and fissures, disruption of the cellular membrane, clumping of cells, leakage of intracellular components and breakdown of entire cells. It may give rise to the leakage of nutrients and genetic components (*6,25*). Only *Listeria monocytogenes* ATCC 13932 treated with ethanolic lipid fraction of bay leaf at 500 μg/mL showed minor changes in cellular morphology. When comparing the results of antibacterial evaluation (time-kill assay, cell constituent release and SEM) against two *Vibrio* species tested, it could be depicted from the data that lipid fractions were more potent against *Vibrio alginolyticus* ATCC 17749. These results strongly support the use of these lipid fractions to prevent *Vibrio alginolyticus* contamination in seafood with a useful application in export of such food commodities.

In a nutshell, lipid fractions obtained from bay leaf could be a potent antibacterial agent to inhibit all the tested pathogens *in vitro*. Bay leaf is a commonly used condiment in South Asian cuisines. If this herb lipid fraction is supplemented to food preparations, it will be able to combat *Listeria monocytogenes*, which is a common food contaminant that causes serious foodborne illnesses, and also enhance sensorial attributes of the food items.

Table 2. The effect of lipid fractions on cell constituent release of the tested foodborne pathogens measured as absorbance at 260 nm

Foodborne pathogen	$A_{260\,nm}$								
	t(incubation)=1 h								
	Control	EC	MC	EN	MN	EB	MB	EF	MF
Vibrio parahaemolyticus ATCC 17802	(0.51±0.02)^a	(0.72±0.02)^b		EN (0.71±0.1)^{ab}			MB (0.74±0.05)^c		
Vibrio alginolyticus ATCC 17749	(0.50±0.02)^c	(0.72±0.03)^{bc}		EN (0.70±0.01)^{ab}	MN (0.71±0.1)^{ab}	EB (0.76±0.01)^c		EF (0.70±0.03)^a	
Bacillus cereus ATCC 11778	(0.53±0.01)^a	(0.70±0.01)^b		EN (0.72±0.3)^b		EB (0.73±0.02)^b	MB (0.73±0.04)^b		MF (0.71±0.07)^b
Listeria monocytogenes ATCC 13932	(0.53±0.11)^a	(0.72±0.01)^{bc}	MC (0.71±0.07)^b	EN (0.71±0.03)^b	MN (0.72±0.03)^{bc}	EB (0.72±0.01)^{bc}	MB (0.73±0.01)^c	EF (0.73±0.01)^{bc}	MF (0.72±0.01)^{bc}
Escherichia coli ATCC 8739	(0.54±0.03)^a	(0.70±0.04)^b	MC (0.71±0.11)^b	EN (0.65±0.01)^b	MN (0.69±0.09)^b	EB (0.70±0.01)^b	MB (0.7±0.03)^b		
	t(incubation)=2 h								
	Control	EC	MC	EN	MN	EB	MB	EF	MF
Vibrio parahaemolyticus ATCC 17802	(0.53±0.07)^a	(0.73±0.06)^b		EN (0.72±0.05)^b			MB (0.74±0.04)^b		
Vibrio alginolyticus ATCC 17749	(0.52±0.17)^a	(0.74±0.02)^b		EN (0.73±0.05)^b	MN (0.73±0.03)^b	EB (0.75±0.03)^b		EF (0.73±0.06)^b	
Bacillus cereus ATCC 11778	(0.55±0.10)^a	(0.71±0.10)^{ab}		EN (0.73±0.03)^{ab}	MN (0.73±0.03)^b	EB (0.84±0.10)^c	MB (0.80±0.05)^c		MF (0.73±0.10)^b
Listeria monocytogenes ATCC 13932	(0.57±0.01)^{abc}	(0.73±0.06)^{abc}	MC (0.70±0.11)^a	EN (0.71±0.15)^{abc}	MN (0.75±0.03)^{abc}	EB (0.76±0.13)^{cd}	MB (0.90±0.24)^d	EF (0.76±0.04)^{bc}	MF (0.74±0.13)^{abc}
Escherichia coli ATCC 8739	(0.56±0.10)^b	(0.75±0.01)^b	MC (0.74±0.05)^{ab}	EN (0.78±0.11)^{bc}	MN (0.75±0.06)^{bc}	EB (0.80±0.22)^c	MB (0.74±0.11)^b		

Results are mean values of triplicates±S.D. Values in the same column with different superscript are significantly different at $p<0.05$. MN, MF, MC, MB, EN, EF, EC and EB stand for methanolic and ethanolic extracts of lipid fractions of black cumin, fennel seeds, coriander seeds and bay leaf, respectively, at minimum inhibitory concentrations (MICs)
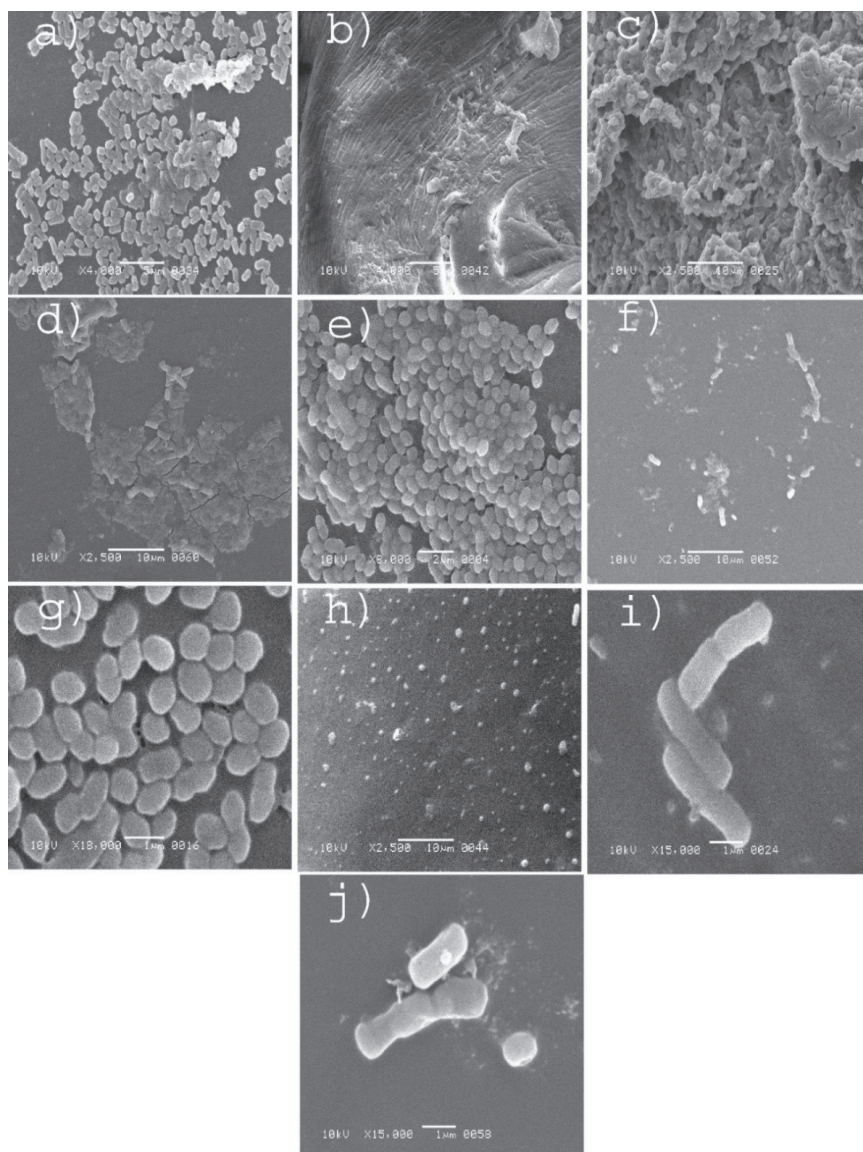
Fig. 6. Scanning electron microscopic images of tested bacteria when treated with lipid fractions at ½ minimum inhibitory concentrations (MICs). *Escherichia coli* ATCC 8739: a) control, b) treated with ethanolic extract of lipid fraction of black cumin at γ=125 µg/mL; *Bacillus cereus* ATCC 11778: c) control, d) treated with ethanolic extract of lipid fraction of black cumin at γ=125 µg/mL; *Vibrio alginolyticus* ATCC 17749: e) control, f) treated with ethanolic extract of lipid fraction of black cumin at γ=125 µg/mL; *Vibrio parahaemolyticus* ATCC 17802: g) control, h) treated with ethanolic extract of lipid fraction of coriander at γ=500 µg/mL; and *Listeria monocytogenes* ATCC 13932: i) control, j) treated with ethanolic extract of lipid fraction of bay leaf at γ=500 µg/mL

The results presented in this study are novel due to several reasons: there is no report on the lipid fractions from selected herbs/spices from this geographical location reported here extracted by the solvent extraction method used in this study; the antibacterial potency of these lipid fractions was inhibitory even in their lowest tested concentrations against all the selected food pathogens; there are no reports on time-kill studies of such lipid fractions (including for *Vibrio* species); these novel results also confirm the possibility of using these lipid fractions in food matrices that are likely to be contaminated by tested pathogens; there is no previously published report which discusses the time-kill assay against selected *Vibrio* species, and there is no report on the antibacterial action in such low concentration used in this research, *i.e.* 62.5 µg/mL.

CONCLUSION

The antibacterial activity of eight different lipid fractions against five foodborne pathogens was determined and equated in this research. The lipid fraction of coriander seed extracted by methanol showed the lowest total viable count when assayed against *Listeria monocytogenes* ATCC 13932 after 24

h of incubation. The evaluation of cellular constituent release showed that lipid fractions disrupted the integrity of cellular envelope, causing the lysis of microbes with the highest values observed for *Listeria monocytogenes* ATCC 13932 when treated with methanolic extract of lipid fraction of bay leaf. SEM images also illustrated the antibacterial potential of the tested lipid fractions at 18 h of incubation. In conclusion, lipid fractions can effectively function against both tested Gram-positive and Gram-negative isolates even at lowest concentrations. More explanatory research is required to determine the potential of the selected lipid fractions in food matrices.

ACKNOWLEDGMENTS

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