



# Antibacterial efficacies and time-kill kinetics of indigenous Ghanaian spice extracts against *Listeria monocytogenes* and some other food-borne pathogenic bacteria

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## ARTICLE INFO

### Keywords:

Dietary spices  
Antibacterial activity  
Time-kill kinetics  
*L. monocytogenes*  
GC-MS  
Ghana

## ABSTRACT

Increase in food-borne outbreaks has become public health concern worldwide. Exploitation of the antimicrobial properties of dietary spices has become important pharmaceutical tool for controlling food-borne pathogens. This study aimed at evaluating the antibacterial potentials of Ghanaian spices against *Listeria monocytogenes* and other prevalent food-borne pathogens. In preliminary studies, *Listeria* was isolated from some food samples. The overall prevalence of *Listeria* spp. was 23% (13/56). Of the 56 samples examined, 7% showed pathogenic potential for *L. monocytogenes*. Different solvent extracts of thirteen spices namely Calabash nutmeg, West African black pepper, Aidan, Grains of paradise, Negro pepper, Aniseed, African locust bean, Cinnamon, Black pepper, Clove, Cayenne, Basil, and Rauwolfia were tested for their potentials to inhibit clinical and isolated strains of *L. monocytogenes* using qualitative and quantitative antimicrobial assay methods. Only clove and negro pepper among the thirteen different solvent extracts showed bacteriostatic and bactericidal activity against *L. monocytogenes* indicated by minimum inhibitory concentrations ranging from 0.05% to 0.4% and minimum bactericidal concentrations ranging from 0.1% to > 0.4% under experimental conditions. Time-kill study demonstrated listericidal activity of ethanolic clove and negro pepper extracts indicated by absolute mortality of more than 3 log units at 2x MIC and 4x MIC. GC-MS analysis revealed three and eight major chemical components present in clove and negro pepper respectively. *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Bacillus cereus*, and *B. subtilis* also showed satisfactory susceptibilities to ethanolic extracts with MIC ranging from 0.025% to > 0.8%. In general, negro pepper showed broad activity eliciting inhibitory effects against all the tested pathogens. The findings suggest that clove and negro pepper may be promising antibacterial candidates for the decontamination and control bacterial pathogens in food and food supply chain.

## 1. Introduction

*Listeria monocytogenes* is a Gram-positive pathogenic bacterium implicated in listeriosis, a severe food-borne illness characterized by septicemia, perinatal infections, meningitis, spontaneous abortion, and gastroenteritis (Barre et al., 2016). The illness has high fatality rate ranging from 30% to 75%, particularly affecting elderly people, newborns, pregnant women, and immunocompromised individuals. The pathogen is mostly transmitted through fecal-oral route (Nayak et al., 2015) and has been isolated from various foods, including meat, fish and seafood products, raw and pasteurized milk products, salads, fruits, and

vegetables (Szymczak et al., 2020). It can grow at chilling temperatures and has biofilm forming ability on both biological and non-biological surfaces. It is tolerant to osmotic stress (Osaili et al., 2011), which makes its elimination difficult in the food industry and ultimately causing cross-contamination. *Listeria* spp. contribute significantly to global food-borne diseases. Approximately, 48 million people after consuming contaminated food develop various symptoms of illness, with 128,000 hospitalization and 3,000 deaths annually; of which 1,600 are listeriosis cases that results in about 260 deaths (Moura et al., 2016).

With increasing number in the cases of listeriosis, health officials are developing strategies to suppress the growth of *Listeria* in food. Also,

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food-borne pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae* among others, are contributing factors to global resurgence in the outbreaks of food-borne illnesses. Food industries, with the development of advanced processing techniques, apply chemical antibacterial preservatives (Chen et al., 2019) such as sodium benzoates, nitrates, nitrites, etc., to retard the proliferation of some of these bacterial pathogens in food to increase the shelf life (Wang et al., 2018). However, these preservatives are not widely accepted by consumers due to perceived harmful effects on the health of human populace as for instance, the implicated serum glycine decline and carnitine deficiency associated with sodium benzoates (Lennerz et al., 2015), the risk of gastric cancer associated with nitrates and nitrites (Song et al., 2015), and other effects that may be related.

Antibiotics emerged at around 1930s and have been used extensively to control food-borne bacterial infections in clinical practice (Chen et al., 2019). Despite the massive effort to control bacterial infections, evolutionary transformation and variation have led to the emergence of bacterial resistance to know antibiotics (Costa et al., 2017). Molecular research has shown that resistance to antibiotics emanated from the acquisition and dissemination of exogenous genes through genomic islands and mobile genetic elements (Partridge et al., 2018) which can be integrated into bacterial genomes and resistance cassettes leading to cumulative drug resistance (Cerezales et al., 2020). The development of antibiotic resistance and side effects of some antibiotics and chemical preservatives are pressing factors triggering the search for alternative antibacterial agents. Novel alternative agents could include the use of better selectivity and low toxicity antimicrobial peptides. Despite these advantages, short plasma half-life, oral bioavailability, and high cost of production have limited their applications (Liscano et al., 2020; Otvos and Wade, 2014). Furthermore, bacteriophage endolysins have also emerged as an alternative. However, extra measures to avoid contamination, higher cost of production, and the great deal of time needed to grow host bacterial strains in high volumes to produce high yield of phage are a cumbersome concept (Murray et al., 2021). Focus is now on natural antimicrobials among plant origin for food preservation. Thus, the investigation of certain plant species for their antimicrobial capacities as ideal candidates to explore novel antibacterial drugs may yield useful results even against antibiotic-resistant bacterial strains.

Naturally based antibacterial agents such as culinary spices are potential alternatives. They have been applied to food since ancient times, not only as preservatives or flavoring agents, but also used in folk traditional system of medicine (Shan et al., 2007). Additionally, they are Generally Recognized as Safe (GRAS) for human consumption. They possess biologically active compounds acting as antimicrobials, antioxidants, and anticarcinogens. The biological and medical effects of these plant origins are mainly due to the intricate products of secondary metabolism. Among these metabolites, terpenoids quinones, phenolics and phenolic acids, lectins, polyacetylenes, alkaloids, tannins, and flavonoids (Nabavi et al., 2015; Witkowska et al., 2013) are known to be antimicrobial agents which are involved in multiple antimicrobial actions such as cell wall damage, inhibition of extracellular enzymes (Takó et al., 2020), leakage of intracellular components, modification of fatty acids, destruction of protein translocation as well as changes in the synthesis of DNA and RNA (Ceruso et al., 2020; Shan et al., 2007; Witkowska et al., 2013) when exposed against microorganisms.

In Ghana, spices are used in a variety of ways such as flavorings in traditional drinks, seasonings, ingredients for cooking, and in folkloric medicine to treat female infertility, hemorrhoids, and epilepsy (Abbiw, 1990). Recently, there has been a considerable emphasis on studies involving spices as means of controlling bacterial pathogens (Kayira and Nakano, 2020; Mostafa et al., 2018; Sharma et al., 2017). While there is a growing knowledge on antimicrobial efficacy of spices against food-borne bacterial pathogens, there is limited information on Ghanaian spices against such pathogens. Furthermore, *L. monocytogenes* as an emerging food-borne pathogen in Ghana has not been tested with some plant materials to any extent before. Accordingly, the purpose of

this study was to investigate antibacterial efficacies of different solvent extracts of 13 Ghanaian spices against isolated and clinical strains of *L. monocytogenes*. The extracts were also tested against *S. aureus*, methicillin-resistant *S. aureus*, *Bacillus cereus*, *B. subtilis*, which are also prevalent food-borne pathogens of importance.

## 2. Materials and methods

### 2.1. Acquisition and extraction of plant material

Thirteen plant materials (spices) locally cultivated in Ghana (Ghanaian origin) were purchased from popular spice markets in March 2019. Before sample collection, a preliminary survey was conducted with the spice dealers who had close contact with the farmers in the various regions to authenticate and ascertain the source of the spices. Spices were chosen based on their widespread use in local community (use for cooking and use in folk medicine). The collected samples were disinfected, air dried in a shade and pulverized to increase the surface area. Each sample was weighed and soaked in different extraction solvents (99.5% ethanol, 99% methanol, and 99% dimethyl sulfoxide DMSO; Nacalai tesque, INC, Kyoto, Japan) in the ratio 1:9 w/v. The mixtures were subjected to shaking at 25 °C for 48 h. For distilled water extracts, weighed samples were soaked in 80 °C distilled water for 30 min with intermittent shaking. Filtration was performed using Whatman filter paper (110 mm; Toyo Roshi Kaisha, Ltd, Japan) and the filtrate subsequently centrifuged at 9500 rpm for 15 min at 4 °C. The supernatants (10% extracts) were obtained and stored at 10 °C until use.

### 2.2. Isolation of *Listeria* from food

#### 2.2.1. Sample collection

Three categories of food sample were considered in this study. A total of 56 food samples, comprising of raw chicken meat (n = 18), some Ready-to-Eat (RTE) food (sandwich, vegetable salad, coleslaw, maze-soba, tuna macaroni salad, grilled sausage, grilled pork, sushi, cooked fish in sauce, grilled fish, and sprout, n = 27), and some dairy products (cheese, pasteurized milk, yoghurt, and milk powder, n = 11) were randomly sampled from eight retail facilities in Higashi-Hiroshima, Japan from July 2019 to October of the same year. Three samples each of raw chicken and RTE, and two or one dairy product were randomly sourced on different occasions from different sites, and then transported on ice packs to the laboratory for microbiological examination. The samples were selected based on commonly known foods implicated in the spread of *Listeria*.

#### 2.2.2. Enrichment and isolation performances

ISO 11290-1:2017 method involving a double enrichment procedure was employed to isolate *Listeria* species from the various food categories. Briefly, primary enrichment of 25 g test portion of each sample was done in 225 mL liquid broth, containing reduced concentration of enrichment and selective supplements (half-strength Fraser broth) at 30 °C for 24 h. This was followed by secondary enrichment of 0.1 mL aliquot obtained from primary enriched culture in 10 mL broth, containing full concentration of enrichment and selective supplements (full-strength Fraser broth) at 37 °C for 48 h. For liquid samples, 10 mL test portion was employed in 90 mL of half-strength Fraser broth followed by second enrichment. One loop full of each secondary enriched broth cultures was surface streaked on two selective agar plates (ALOA and PALCAM) followed by incubation at 37 °C for 24–48 h. Characteristic blue-green colonies surrounded by opaque halo on ALOA and gray-black colonies surrounded by diffuse halo on PALCAM were obtained for purification on tryptic soy yeast extract agar (TSYEA) for identification tests.

#### 2.2.3. Phenotypic and biochemical profile

Various mandatory confirmation tests were performed on isolated colonies from TSYEA following the standard to ascertain the phenotypic

and biochemical characteristics of the isolates. The Gram's staining was performed followed by microscopic examination and catalase test. Characteristic Gram-positive short rods or coccobacilli which showed effervescence upon addition of 3% hydrogen peroxide (catalase positive) were sub-cultured in non-selective Brain Heart Infusion (BHI) medium at 25 °C for 18–24 h.

Motility was performed by spotting a drop of BHI culture on a sterile glass slide followed by examination under a compound microscope. Typical *Listeria* species exhibited tumbling motility and are short rods. To confirm the results, an alternative test for motility was performed. Briefly, semi-solid nutrient agar tubes containing 0.4% agar were stabbed with an inoculating needle and incubated at 25 °C for 5 days and motility observed. *Listeria* species were motile as observed by umbrella-like growth patterns and growth away from the stabbed line.

Furthermore, the isolates were subjected to Voges-Proskauer and sugar fermentation tests. For Voges-Proskauer test, 3 mL tubes of VP medium were inoculated and incubated at 37 °C for 24 h. After incubation, 3 drops each of 5%  $\alpha$ -naphthol and 40% potassium hydroxide solution were added and allowed to stand for 15 min. A positive reaction was indicated by red band layer on top of the medium. Typical *Listeria* species are VP-positive.

Sugar fermentation test was performed in test tubes containing bromocresol blue agar base supplemented with 5% each of L-rhamnose, D-xylose, D-glucose, and D-mannitol. The tubes were inoculated with TSYEA cultures and incubated at 37 °C for 24–28 h. Fermentation of sugar (acid formation) was indicated by change in color from blue to yellow.

#### 2.2.4. Pathogenicity confirmation test with hemolytic *S. aureus*

Christie Atkins Munch Peterson (CAMP) test was performed to observe the degree of hemolysis on Columbia agar supplemented with 5% sheep blood. The agar was streaked with  $\beta$ -hemolytic *S. aureus* (209P) and then with patterns of perpendicular streaks with *Listeria* isolates following incubation at 37 °C for 24 h. Positive reaction was indicated by development of  $\beta$ -hemolysis at the intersection of *Listeria* isolates with *S. aureus* showing synergistic effect of haemolysins for CAMP positive reaction.

### 2.3. Antibacterial assay

#### 2.3.1. Test bacterial strains and growth conditions

The test pathogens were clinical Gram-positive isolates obtained from Hiroshima City Institute of Public Health (HCIPH), Hiroshima, Japan. They include *L. monocytogenes* (*L. m.* AS-1; serotype 4b), *L. monocytogenes* (*L. m.* M24–1; serotype 1/2c), *B. cereus* (IFO 3457), *B. subtilis* (BF), *S. aureus* (209P), and methicillin-resistant *S. aureus* (MRSA). Four *L. monocytogenes* strains (*Lis* C1, *Lis* C2, *Lis* C3, and *Lis* C4) isolated from raw chicken meat in this study as described above were also included. All *L. monocytogenes* strains were cultivated in tryptic soy broth or agar (TSB, TSA, Eiken Chemical Co., Ltd, Japan). The assay medium used for growing the remaining bacteria strains was nutrient broth or agar (NB, NA, Eiken Chemical Co., Ltd, Japan). A single isolated colony from 24 h old TSA or NA plates was suspended in 10 mL of appropriate broth medium and incubated aerobically at 35 °C for 24 h under static condition. Prior to antibacterial testing, turbidity of inoculum was standardized to match 0.5 McFarland standard. Briefly, 1 mL aliquot broth culture was diluted in sterile TSB or NB medium to final concentration of  $10^6$  CFU/mL ( $OD_{620} = 0.08–0.1$ ).

#### 2.3.2. Screening approach for antibacterial activity

The various solvent extracts (10%) were used for evaluation of antibacterial activity against *L. monocytogenes* employing Kirby-Bauer disk diffusion method as described by Klančnik et al. (2010) with minor modifications. Only ethanolic extracts were tested against *S. aureus*, MRSA, *B. cereus*, and *B. subtilis*. Ten mL of molten TSA or NA was poured into a sterile petri dish to form a base layer. The base layer

was seeded with 5 mL of agar previously inoculated with the bacterial culture. Sterilized paper disks (8 mm in diameter; Tokyo Roshi kaisha, Ltd. Japan) impregnated with 50  $\mu$ L of each extract were placed on the surface of each agar. The plates were kept in the fridge at 10 °C for 2 h to allow diffusion of active agents into the agar then incubated at 35 °C for 24 h. Extraction solvents with their respective concentrations served as negative controls. The antibacterial efficacies of the extracts were indicated by clear zone of inhibition surrounding the paper disk. Experimental replicates were established for three independent runs and diameter of inhibition zones computed as means  $\pm$  standard deviations.

#### 2.3.3. Determination of minimum inhibitory concentrations (MICs)

The agar dilution method was employed to determine MIC as described by Klančnik et al. (2010) with minor modifications. Briefly, varying concentrations of each spice extract were diluted in 20 mL molten TSA or NA tempered to 52 °C to obtain a desired final concentration of 0.025%, 0.05%, 0.1%, 0.2%, and 0.4%. Ethanolic extracts were tested up to 0.8% for all other pathogens except *L. monocytogenes*. Bacterial cultures ( $10^6$  CFU/mL) were streaked on solid TSA (*L. monocytogenes*) or NA (*S. aureus*, *B. cereus*, *B. subtilis*, and MRSA). Agar plates were incubated under aerobic condition at 35 °C for 24 or 48 h. The MIC was defined as the lowest concentration of the extracts in agar that showed no observable growth (i.e., perfect inhibition) after 24 or 48 h. Extraction solvents in amounts corresponding to highest concentration (4% or 8%) in extract diluted assay and inoculated agar plates without added spice extracts were used as negative controls.

#### 2.3.4. Determination of minimum bactericidal concentrations (MBCs)

The macro dilution broth method as described by Kayira and Nakano (2020) was used to determine MBC with some adjustments. A set of test tubes holding 10 mL of TSB or NB were diluted with different concentration of the various solvent extracts to a desired final concentration of 0.025%, 0.05%, 0.1%, 0.2%, and 0.4%. Ethanolic extracts were tested up to 0.8% for all other pathogens except *L. monocytogenes*. Each concentration was inoculated with bacterial cultures ( $10^6$  CFU/mL) and then incubated at 35 °C for 24 h. Control experiments were broth test tubes containing 4% or 8% solvent and those without added plant extract. After incubation, the preparations were sub-cultured by spreading 0.1 mL aliquot on solid TSA or NA plates and incubated at 35 °C for 24 h and examined for bacterial growth. The MBC was defined as the lowest concentration of the extract that demonstrated 99.9% or 100% reduction of the bacterial growth (Das et al., 2010; Sharma et al., 2012).

#### 2.3.5. Time-kill kinetics assay

The killing rate and the extent of antibacterial action by ethanolic spice extracts against *L. monocytogenes* was determined by time-kill kinetics assay method previously described by Bag et al. (2012) with minor modifications. Briefly, overnight TSB cultures (24 h) of cocktail of clinical *L. monocytogenes* (4b and 1/2c) strains were washed three times by centrifugation at 1000 rpm for 5 min in physiological Phosphate Buffer Saline (PBS) pH 7.4. The cells were harvested, resuspended in TSB, and exposed to concentrations equal to 0.5x MIC, 1x MIC, 2x MIC, and 4x MIC of clove and negro pepper extracts. Samples were taken at 0, 2, 4, 6, 8, 10, and 24 h and serially diluted in PBS and spread-plated. The plates were incubated at 37 °C for 24 h. Inoculum without spice extract was plated as control. The procedure was repeated in three independent runs for each extract and the means expressed as  $\log_{10}$  CFU/mL. Time-kill curves were generated by plotting  $\log_{10}$  CFU/mL against time. The decrease in  $\log_{10}$  CFU/mL > 3 log units indicate listericidal effect of the extracts.

### 2.4. GC-MS analysis of antilisterial extracts

Ethanolic extracts of clove and negro pepper were analysed for their chemical constituents using modified GC-MS analysis method (Yamuna

et al., 2017) on equipment model JMS-T100GCv "AccuTOF GCv 4G" (JEOL Ltd., Tokyo, Japan) equipped with HP-5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). For GC-MS spectroscopic detection, an electron ionization system with ionization energy of EI+ (70 eV, 300 µA) was used. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 mL/min. Ion source temperature and injector temperature were set to 250 °C and 300 °C, respectively. GC oven temperature was programmed from 50 °C to 150 °C and then held isothermally for 10 min and finally raised to 300 °C at 10 °C/min. 1 µL of diluted sample (1/100, v/v, in ethanol) was injected into the column in a split mode with a split ratio 10:1.

#### 2.4.1. Identification of chemical constituents

The chemical compounds in the extracts were identified based on GC retention times on the HP-5 capillary column. Interpretation of the mass spectra were conducted using the database of National Institute of Standards and Technology (NIST) having more than 62,000 patterns. The name, molecular formula, molecular weight, and structure of the components were ascertained. The relative percentage of the chemical constituents in the extracts were expressed as percentage by peak area normalization.

#### 2.5. Statistical analysis

Statistical computations for DIZ data were performed using IBM SPSS Version 25.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as means of three replicates ± standard deviation (SD) and subjected to one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was employed to discriminate between the means whenever significant difference existed using 5% ( $p < 0.05$ ) level of confidence.

### 3. Results

#### 3.1. Prevalence and phenotypic profiles of *Listeria* spp. in various food categories

The overall prevalence of *Listeria* spp. was 23% (13/56) as recorded in Table 1. The prevalence of *Listeria* spp. was predominantly recorded in raw chicken meat samples (56%, 10/18) compared to RTE (7%, 2/27) and dairy products (9%, 1/11). Among the species identified, *L. innocua* was the most prevalent (18%, 10/56) followed by *L. seeligeri* (5%, 3/56) and *L. marthi* (5%, 3/56). *L. monocytogenes* was prevalent in 7% (4/56) of the samples with all positive isolates obtained from raw chicken meat. Two unknown isolates (Table 2) recording a prevalence of 4% (2/56) were also identified.

#### 3.2. Ethnobotanical information of plant species

The ethnobotanical data of utilized spices and their extract pH are recorded in Table 3. These constitute thirteen plant species among 10 families representing good diversity of spices used in this study. The medicinal and biological properties of these plant species are evident owing to the diverse use in folkloric medicine and as agents for controlling bacterial infections. Various plant extract exhibited varying pH levels ranging from 4.35 to 7.56.

**Table 1**

Prevalence and distribution of *Listeria* spp. in various food categories.

Sample category	No. of samples	%	No. of samples positive for <i>Listeria</i> spp. (%)					Total	
			<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. ivanovii</i>	<i>L. seeligeri</i>	<i>L. marthi</i>		Unknown
Raw chicken	18	10 (56)	4 (22)	10 (56)	1 (6)	2 (11)	3 (17)	0 (0)	20*
RTE	27	2 (7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (7)	2
Dairy product	11	1 (9)	0 (0)	0 (0)	0 (0)	1 (9)	0 (0)	0 (0)	1
Overall	56	13 (23)	4 (7)	10 (18)	1 (2)	3 (5)	3 (5)	2 (4)	23 * (41)

\*: repeated number of samples, RTE: Ready-To-Eat food

#### 3.3. Screening approach for antimicrobial activity

All the 13 different solvent spice extracts were screened for their antimicrobial activities against *L. monocytogenes* using disk diffusion method. The antimicrobial activities are presented as diameter of inhibition zones (DIZ) in Tables 4 and 5. The results revealed that the extracts have significant ( $p < 0.05$ ) but variable potency in suppressing microbial growth. Only ethanol and methanol extracts of negro pepper among the different extracts demonstrated inhibitory activity against *L. monocytogenes* with DIZ ranging from 9.0 mm to 10.3 mm. Similarly, ethanol, methanol, and DMSO extracts of clove showed significant ( $p < 0.05$ ) antibacterial efficacy against *L. monocytogenes* with DIZ ranging from 10.8 mm to 13.3 mm. Moreover, distilled water extract of clove revealed satisfactory activity (9.5–11.2 mm) against the test pathogens except *Lis* C1 and *Lis* C3 isolates from raw chicken meat. Surprisingly, DMSO and distilled water extracts of negro pepper elicited non-inhibitory activity against the pathogens at the tested concentration (Table 4).

In Table 5, the DIZ of ethanolic spice extracts against food-borne *S. aureus*, MRSA, *B. cereus*, and *B. subtilis* are shown. The pathogens responded considerably with different degrees of susceptibility to the extracts with DIZs ranging from 8.3 mm to 16.0 mm. Ethanol extracts of negro pepper showed significant ( $p < 0.05$ ) zone of inhibition, particularly against *S. aureus* and MRSA with DIZs of 16 mm and 15.3 mm, respectively. Results of antimicrobial activity showed that spice extracts have varying degrees of effectiveness against bacterial pathogens. *S. aureus* and MRSA responded better compared to *B. cereus* and *B. subtilis* which demonstrated most resistance to the extracts at the tested concentration when disk diffusion assay method was employed.

#### 3.4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Disk diffusion assay may not be a reliable method to conclude the antimicrobial activity of spice extract. Thus, MIC and MBC assays were employed to quantitatively evaluate antibacterial activity of extracts to set up their bacteriostatic and bactericidal concentrations.

Using the macro dilution broth and agar dilution methods the ability of bacterial pathogens to produce visible growth at certain concentrations was investigated of all spice extracts used in the screening assay. The MIC and MBC values as determined by agar and broth dilution methods respectively are illustrated in Tables 6 and 7. Table 6 shows listeristatic and listericidal concentrations of spice extracts prepared from four different extraction solvents. Of all the different extracts, only clove and negro pepper demonstrated ability to suppress the growth of *L. monocytogenes* with MIC ranging from 0.05% to 0.4% and MBC ranging from 0.1% to > 0.4%. Clove and negro pepper extracts suppressed the growth *L. monocytogenes* mostly at MIC of 0.2%, while 0.4% (MBC) was adequate to result in total mortality. Moreover, at the same MBC concentration (0.4%), ethanolic clove and negro pepper extracts did not exert bactericidal effect against *L. monocytogenes* (*Lis* C3) isolate obtained from raw chicken. Meanwhile, some solvent extracts revealed that bactericidal concentrations are enough to elicit a killing effect. For instance, methanol extracts of clove at inhibitory concentration of 0.2% demonstrated bactericidal efficacy against *L. m.* AS-1 (4b) and *L. m.*

**Table 2**  
Phenotypic, biochemical and pathogenicity profiles of isolated *Listeria* spp.

Identified <i>Listeria</i> spp.	Phenotypic profile*					Biochemical and Sugar fermentation*					Pathogenicity profile*	
	MP	GR	MTB	MTA	CT	VP	LR	DX	MN	GL	βH	CS
<i>L. monocytogenes</i>	Rod	+	+	+	+	+	+	-	-	+	+	+
<i>L. innocua</i>	Rod	+	+	+	+	+	+	-	-	+	-	-
<i>L. ivanovii</i>	Rod	+	+	+	+	+	-	+	-	+	+	-
<i>L. seeligeri</i>	Rod	+	+	+	+	+	-	+	-	+	+w	+
<i>L. marthi</i>	Rod	+	+	+	+	+	-	-	-	+	-	-
Unknown	Rod	+	+	+	+	-	-	-	d	d	-	-
Unknown	Rod	+	+	+	+	-	-	-	-	d	+	+

\*MP: Morphology, GR: Gram's reaction, MTB: Motility by microscopy, MTA: Motility in semi-solid gar, CT: Catalase test, VP: Voges-Proskauer, LR: L-Rhamnose, DX: D-Xylose, MN: Mannitol, GL: Glucose, βH: β-hemolysis, CS: Chris Atkins Munch Peterson test with *S. aureus*, + : positive, - : negative, + w: weak, d: variable.

**Table 3**  
Ethnobotanical classification of utilized spice species and their extract pH.

Plant species	Family	Common name	Local Ghanaian name	Part used	Extract pH	Biological action/Medicinal uses
<i>Monodora myristica</i>	Annonaceae	Calabash nutmeg	Wediaba	Seed	6.45	Inhibitory effects on fungi ( <i>Aspergillus</i> spp., <i>Rhizopus</i> spp.) and bacteria ( <i>B. Subtilis</i> , <i>S. aureus</i> ) (Akise et al., 2020) Treat elevated cholesterol levels (Nwoso et al., 2015).
<i>Piper guineense</i>	Piperaceae	West African black pepper	Esrowisa	Fruit	7.24	Treat sickle cell anemia (Freiesleben et al., 2015).
<i>Tetrapleura tetraptera</i>	Fabaceae	Aidan	Prekese	Fruit	6.91	Molluscidal activity, anti-convulsant, attenuates high carbohydrate (Kuate et al., 2015).
<i>Aframomum melegueta</i>	Zingiberaceae	Grains of paradise	Fomwisa	Fruit/Seeds	5.98	Repellent activity against <i>Sitophilus zeamais</i> , antigrowth against tumor cells (Freiesleben et al., 2015; Ukeh et al., 2009).
<i>Xylopiya aethiopicum</i>	Annonaceae	Negro pepper	Hwentia	Fruit	5.51	Antiseptic potentials, arrests bleeding, and antifungal efficacies (Konning et al., 2004; Ogbonnia et al., 2008). Inhibitory activity against <i>B. subtilis</i> , <i>S. aureus</i> , <i>Pseudomonas aeruginosa</i> (Fleischer et al., 2008).
<i>Pimpinella anisum</i>	Apiaceae	Aniseed	Nketekete	Seed	5.43	Treat epilepsy and seizure, antidiabetic, gastro protective (Shojaii and Abdollahi Fard, 2012; Sun et al., 2019).
<i>Parkia biglobosa</i>	Fabaceae	African locust bean	Dawadawa	Seed	7.40	Immunomodulating properties (Zou et al., 2014). Inhibitory effects against bacteria (Udobi and Onaolapo, 2009)
<i>Cinnamomum zeylanicum</i>	Lauraceae	Cinnamon	Cinnamon	Bark	4.40	Antimicrobial activity against <i>Helicobacter pylori</i> (Ali et al., 2005) Diabetes, Excessive menstruation and backache (Kim et al., 2006; Lee and Balick, 2005).
<i>Piper nigrum</i>	Piperaceae	Black pepper	-	Fruit	4.35	Antioxidant, radical scavenging, bactericidal, anti-adhesive activity, treat flu and muscle rushes (Chen et al., 2019; Gülçin, 2005; O'Mahony et al., 2005).
<i>Syzygium aromaticum</i>	Myrtaceae	Clove	Pepre	Flowers/Buds	5.12	Antibiofilm activity against <i>P. aeruginosa</i> (El-Abed et al., 2011). Antiviral activity against HSV-1 and HSV-2 viruses (Kamatou et al., 2012).
<i>Capsicum annuum</i>	Solanaceae	Cayenne	Meko	Fruit	5.53	Anti-inflammatory and carminative activity (Pandey et al., 2012).
<i>Ocimum basilicum</i>	Lamiaceae	Basil	Akuko besa	Leaf	7.56	Antibacterial effect against <i>S. aureus</i> and <i>B. cereus</i> (Adigüzel et al., 2005; Moghaddam et al., 2011).
<i>Rauwolfia vomitoria</i>	Apocynaceae	Rauwolfia	kakapenpen	Root	6.85	Treatment of infertility, hypertension, malaria and diarrhea (Lembe et al., 2014). Antifilarial activity (Attah et al., 2013).

M24-1 (1/2c) strains. Interestingly, the extracts showed similar patterns of activity against clinical and isolated strains of *L. monocytogenes*. Higher concentrations of distilled water extracts (>0.4%) may be required to elicit inhibitory activity against the test pathogens. Generally, DMSO and methanol extracts of negro pepper showed strong efficacy recording MIC as low as 0.05% and 0.1%, respectively, against the pathogens.

In Table 7, antibacterial activity of ethanolic spice extracts expressed as MIC and MBC against some common Gram-positive pathogens considered in this study are presented. All extracts showed inhibitory effects against *S. aureus*, except aidan. More importantly, negro pepper showed stronger activity against *S. aureus*, with MIC as low as 0.025% followed by West African black pepper and African locust bean, with MIC of 0.05%. Methicillin-resistant *S. aureus* (MRSA) was also susceptible to most of the antimicrobial agents. Of the 13 ethanolic spice extracts, 10 showed considerable effect against MRSA (Table 7). Nevertheless, ethanolic extracts of aidan, African locust bean, and cayenne elicited no bacteriostatic and bactericidal effects against MRSA even at the highest concentration (0.8%) tested. In terms of spore formers, *B. cereus* and *B. subtilis* were suppressed at 0.05% bacteriostatic

concentration of negro pepper but required 12xMIC of the extract to elicit bactericidal effect. MIC and MBC represented as > 0.4% or > 0.8% indicate that the pathogens did not respond to inhibitory and killing effects, respectively, and that they showed visible colonies on agar plates after 24 h incubation at 37 °C.

In general, negro pepper showed broad activity eliciting relatively high inhibitory activities against all the tested food-borne pathogenic bacteria even at the lowest MIC concentration of 0.025% against *S. aureus* while *B. cereus*, and *B. subtilis* were the most resistant strains (Table 7). Although some extracts showed non-inhibitory activity when disk diffusion method employed, it does not necessarily mean the antimicrobial agents were inactive. Agar dilution and broth dilution methods proved to be a reliable approach to determine antimicrobial activity.

### 3.5. Time-kill kinetics

The bacteriostatic and bactericidal effects of most active extracts (clove and negro pepper) against cocktail of *L. monocytogenes* (clinical strains) are illustrated in Fig. 1 (A and B). The results showed that the

**Table 4**

The sensitivity profiles of different solvent spice extracts against *L. monocytogenes*.

Extraction solvent	Spices	Diameter of Inhibition Zone: DIZ (mm)					
		<i>L. m.</i> AS-1 (4b)	<i>L. m.</i> M24-1 (1/2c)	<i>Lis</i> C1	<i>Lis</i> C2	<i>Lis</i> C3	<i>Lis</i> C4
Ethanol	Negro pepper	10.0 ± 0.0 <sup>a</sup>	10.0 ± 0.0 <sup>a</sup>	10.0 ± 0.0 <sup>a</sup>	9.7 ± 0.3 <sup>a</sup>	9.5 ± 0.0 <sup>a</sup>	9.7 ± 0.3 <sup>a</sup>
	Clove	12.1 ± 0.5 <sup>b</sup>	12.2 ± 0.4 <sup>b</sup>	12.4 ± 0.3 <sup>ab</sup>	12.2 ± 0.2 <sup>ab</sup>	12.0 ± 0.3 <sup>b</sup>	12.7 ± 0.3 <sup>a</sup>
DMSO	Negro pepper	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>
	Clove	11.7 ± 0.5 <sup>a</sup>	12.0 ± 0.5 <sup>a</sup>	11.2 ± 0.2 <sup>a</sup>	10.8 ± 1.2 <sup>a</sup>	11.0 ± 1.4 <sup>a</sup>	11.0 ± 1.4 <sup>a</sup>
Methanol	Negro pepper	9.6 ± 0.1 <sup>bc</sup>	10.3 ± 0.7 <sup>a</sup>	9.3 ± 0.1 <sup>bc</sup>	9.0 ± 0.0 <sup>c</sup>	9.8 ± 0.2 <sup>ab</sup>	9.3 ± 0.3 <sup>bc</sup>
	Clove	12.8 ± 0.5 <sup>bc</sup>	10.8 ± 0.2 <sup>d</sup>	13.5 ± 0.2 <sup>a</sup>	12.5 ± 0.2 <sup>b</sup>	13.3 ± 0.3 <sup>ab</sup>	13.2 ± 0.2 <sup>ab</sup>
Distilled water	Negro pepper	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>
	Clove	9.6 ± 0.5 <sup>a</sup>	11.2 ± 0.7 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	9.5 ± 0.5 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	9.5 ± 0.5 <sup>a</sup>

*L. m.* AS-1(4b): *L. monocytogenes* serotype 4b, *L. m.* M24-1(1/2c): *L. monocytogenes* serotype 1/2c, *Lis* C1, *Lis* C2, *Lis* C3 and *Lis* C4 are *L. monocytogenes* isolates recovered from raw chicken meat. Different superscripts in the same row indicate response values are statistically different ( $p < 0.05$ )

**Table 5**

The sensitivity profiles of ethanolic spice extracts against food-borne pathogens.

Spice species	Diameter of Inhibition Zone: DIZ (mm)			
	<i>S. aureus</i> 209 P	MRSA	<i>B. cereus</i> (IFO 3457)	<i>B. subtilis</i> (BF)
Calabash nutmeg	8.0 ± 0.0 <sup>b</sup>	8.0 ± 0.0 <sup>b</sup>	10.7 ± 0.5 <sup>a</sup>	10.7 ± 0.9 <sup>a</sup>
West African black pepper	8.3 ± 0.6 <sup>b</sup>	11.7 ± 0.9 <sup>a</sup>	8.0 ± 0.0 <sup>b</sup>	8.0 ± 0.0 <sup>b</sup>
Aidan	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>
Grains of paradise	10.5 ± 0.6 <sup>a</sup>	8.3 ± 0.5 <sup>b</sup>	8.0 ± 0.0 <sup>b</sup>	8.0 ± 0.0 <sup>b</sup>
Negro pepper	16.0 ± 2.0 <sup>a</sup>	15.3 ± 0.5 <sup>a</sup>	10.2 ± 0.2 <sup>b</sup>	11.2 ± 1.3 <sup>b</sup>
Aniseed	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>
African locust bean	13.9 ± 0.4 <sup>a</sup>	10.0 ± 0.0 <sup>b</sup>	9.0 ± 1.4 <sup>b</sup>	9.0 ± 1.4 <sup>b</sup>
Cinnamon	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>
Black pepper	8.0 ± 0.0 <sup>b</sup>	10.2 ± 0.2 <sup>a</sup>	8.0 ± 0.0 <sup>b</sup>	8.0 ± 0.0 <sup>b</sup>
Clove	12.7 ± 1.1 <sup>a</sup>	11.2 ± 1.2 <sup>a</sup>	8.0 ± 0.0 <sup>b</sup>	8.7 ± 0.9 <sup>b</sup>
Cayenne	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>
Basil	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>
Rauvolfia	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>
Control (99.5% ethanol)	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>	8.0 ± 0.0 <sup>a</sup>

*S. aureus* 209P: *S. aureus* stain 209P, MRSA: methicillin-resistant *S. aureus*, *B. cereus* (IFO 3457): *B. cereus* strain IFO 3457, *B. subtilis* (BF): *B. subtilis* strain BF, Different superscripts in the same row indicate response values are statistically different ( $p < 0.05$ ).

extracts exerted strong activity against *L. monocytogenes*. Concentrations equal to 0.5xMIC and 1xMIC demonstrated inhibitory effects against the pathogen showing no significant increase or reduction in original inoculum size. Nonetheless, in a time dependent manner the extracts demonstrated listericidal effects indicated by total reduction in bacterial populations at 2xMIC within 10 and 24 h respectively for negro pepper and clove. Increasing the concentration to 4xMIC resulted in cell death within 4 h of more than 3 log units for both extracts.

### 3.6. Analysis of chemical constituents

The detected chemical components present in ethanolic extracts according to their retention times and elution order on HP-5 MS fused silica capillary column are presented in Table 8. The identification and characterization established the percent compositions of the major volatile compounds in ethanol extracts. GC-MS analysis revealed three and eight major bioactive compounds in clove and negro pepper, respectively. The major constituents identified in crude clove extract were eugenol representing 72% of the total compound, caryophyllene (10.40%), and phenol, 2-methoxy-4-(2-propenyl)-, acetate (17.15%). Identities of the major chemical compounds in crude extracts of negro pepper based on the peak area and retention times were (1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (13.58%),  $\beta$ -pinene (42.26%), eucalyptol (9.52%), bicyclo [3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene- (3.86%), octatriene, 1,3-trans-5-trans- (3.93%), 1,6-cyclodecadiene, 1-methyl-5-methylene-8- (1-methylethyl)-, [S-(E,E)]- (10.52%), 2-butanone, 3-methoxy-3-methyl- (3.14%), and bicyclo [5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- (13.19%).

## 4. Discussion

Though we did not genotypically characterize *Listeria* isolates, hence a limitation to our study, mandatory biochemical tests recommended by Barre et al. (2016) in the capacity of European Union Reference Laboratory for *Listeria monocytogenes* were performed. Hemolysis test, CAMP test, and sugar fermentation test were additional confirmation test done towards identification of *Listeria* spp. Biochemical identification tests were also employed by El Hag et al. (2020) to confirm *Listeria* spp. The phenotypic profiles of the isolates showed consistency with previously published literature (Barre et al., 2016; den Bakker et al., 2014; Moura et al., 2016; Weller et al., 2015).

The overall prevalence of *Listeria* spp. was 23% (13/56) which is relatively low compared to some other studies that reported higher prevalence (Osaili et al., 2011; Şanlıbaba et al., 2018). Meanwhile, *Listeria* with pathogenic potential is not commonly reported in Japan. Detection of *L. monocytogenes* (prevalence of 7%) in our study may pose serious threat to the public health. The presence of *L. monocytogenes* could be attributed to cross contamination which may have occurred during transportation, poor handling, and contamination from processing equipment.

The increasing concern about food safety has triggered the development of abundant natural antimicrobial agents to control and suppress the growth of food-borne and spoilage microorganisms. In ancient civilization, plant extracts were noteworthy for treating different kinds of diseases and nearly 30% accounted for worldwide sales of pharmaceutical products (Soberón et al., 2007). Recently, several potent antimicrobial agents have been used widely to retard microbial growth with particular emphasis and interest in naturally occurring antimicrobials (Egamberdieva et al., 2021). This study demonstrated the ability of some plant extracts to suppress the growth of bacterial pathogens when certain concentrations of them were introduced.

As shown in Tables 4 and 5, the investigated plant species showed antimicrobial efficacies against food-borne pathogens to some extent. Studies have demonstrated that ethanolic extracts of clove, cinnamon, and black pepper were non-inhibitory against *S. aureus* (Ababutain, 2011) when disk diffusion method was employed. Shan et al. (2007) also

**Table 6**  
Antibacterial activity of different solvent spice extracts expressed as MIC and MBC (%) against *L. monocytogenes*.

Extraction Solvent	Spices	<i>L. m.</i> AS-1 (4b)		<i>L. m.</i> 24-1 (1/2c)		<i>Lis</i> C1		<i>Lis</i> C2		<i>Lis</i> C3		<i>Lis</i> C4	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ethanol	Negro pepper	0.2	0.4	0.2	0.2	0.1	0.2	0.2	0.4	0.2	> 0.4	0.2	0.4
	Clove	0.2	0.4	0.2	0.4	0.2	0.4	0.2	0.4	0.4	> 0.4	0.2	0.4
DMSO	Negro pepper	0.05	0.2	0.05	0.1	0.1	0.2	0.2	0.4	0.2	0.4	0.2	0.4
	Clove	0.2	0.4	0.2	0.4	0.2	0.4	0.2	0.4	0.4	0.4	0.2	0.4
Methanol	Negro pepper	0.1	0.2	0.1	0.2	0.1	0.2	0.4	0.4	0.4	0.4	0.2	0.4
	Clove	0.2	0.2	0.2	0.2	0.2	0.4	0.2	0.4	0.2	0.4	0.2	0.4
Distilled water	Negro pepper	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4
	Clove	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4	> 0.4

MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration.

**Table 7**  
Antibacterial activity of ethanolic spice extracts expressed as MIC and MBC (%) against food-borne pathogens.

Spice species	<i>S. aureus</i> 209 P		MRSA		<i>B. cereus</i> IFO 3457		<i>B. subtilis</i> BF	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Calabash nutmeg	0.4	(-)	0.4	(-)	(-)	(-)	(-)	(-)
West African black pepper	0.05	0.05	0.05	0.05	0.8	(-)	0.8	(-)
Aidan	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Grains of paradise	0.2	0.4	0.2	0.4	(-)	(-)	(-)	(-)
Negro pepper	0.025	0.05	0.05	0.05	0.05	0.6	0.05	0.6
Aniseed	0.4	0.8	0.2	0.8	(-)	(-)	(-)	(-)
African locust bean	0.05	0.4	(-)	(-)	(-)	(-)	(-)	(-)
Cinnamon	0.4	0.8	0.4	(-)	(-)	(-)	(-)	(-)
Black pepper	0.1	0.6	0.2	0.6	(-)	(-)	(-)	(-)
Clove	0.1	0.2	0.1	0.2	(-)	(-)	(-)	(-)
Cayenne	0.2	0.6	(-)	(-)	(-)	(-)	(-)	(-)
Basil	0.2	0.4	0.4	(-)	(-)	(-)	(-)	(-)
Rauvolfia	0.4	0.6	0.4	(-)	(-)	(-)	(-)	(-)

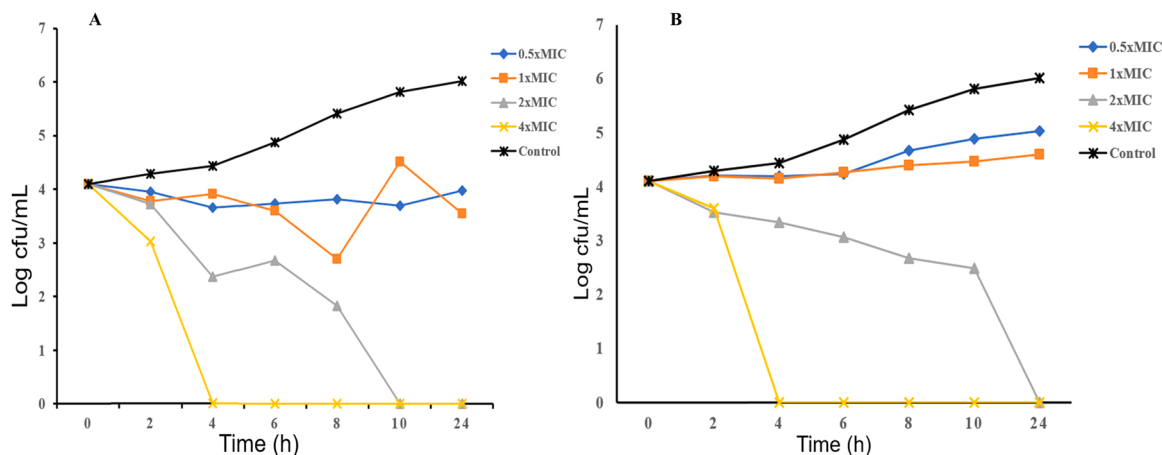
MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration, (-): MIC/MBC (>0.8%) or full growth observed. 8% of various solvents were used as control (full growth)

reported that extract of black pepper had no significant ( $p > 0.05$ ) effects on *B. cereus*, *S. aureus* and *L. monocytogenes* which showed consistency with our results. Furthermore, Adigüzel et al. (2005) reported that ethanolic extract of basil was non-inhibitory against *B. cereus*. Nonetheless there were disparities in the present study compared to other

studies, as for instance, Akthar et al. (2014) observed inhibitory potentials of black pepper against *B. cereus*, and *S. aureus*. Akpomie and Ossai (2011) previously reported DIZ value of 9.0 mm for negro pepper against *S. aureus* whiles Konning et al. (2004) reported comparable DIZ value of 9.8 mm for negro pepper against *S. aureus*. This study revealed higher activity for negro pepper, recording DIZ value of 16.0 mm, compared to previous studies. The different activities of the extracts could be ascribed to differences in geographical locations, maturity of plant species, time of harvest, methodological differences, and chemotype of the extracts.

Hydrophobic properties of natural antimicrobial agents that are poorly soluble or insoluble in water restricts uniform diffusion through agar media (Klančnik et al., 2010). Thus, disk diffusion test may not be appropriate indicator to determine the antimicrobial activity of plants extracts because the agents are separated and partitioned through the agar with respect to their water affinities. The method could be ideal and appropriate for water soluble antibiotics. Previous reports summarized that the absence of inhibition zones does not necessarily mean that a particular agent is inactive especially for non polar compounds which may not or slowly diffuse in culture media (Klančnik et al., 2010; Moreno et al., 2006). Also, the size of inhibition zone is not proportional to the relative effectiveness of antimicrobial compound.

In the quantitative evaluation in agar and broth assays, we established the MICs and MBCs of the extracts to confirm their antimicrobial potentials at varying test concentrations (0.025% to 0.4%) against Gram-positive bacteria. Extracts of negro pepper and clove showed similar trend of activity against *L. monocytogenes* in both qualitative and quantitative methods. *L. monocytogenes* showed susceptibility to only the two extracts with MIC ranging from 0.1% to > 0.4% (Table 6). To some extent, these results were similar to those of previous studies in which ethanolic extracts of clove were potent against *L. monocytogenes* (Bayoub et al., 2010; Cui et al., 2018). No comparable literature was obtained for



**Fig. 1.** Dose and time dependent killing curves of ethanolic extracts of negro pepper (A) and clove (B) against cocktail of clinical *L. monocytogenes* (serogroup 4b and 1/2c).

**Table 8**  
Major chemical compositions of ethanolic antilisterial extracts.

Extract	Retention time (min)	Name of compounds	Molecular formula	Composition (%)
Negro pepper	4.62	(1 R)- 2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	C <sub>10</sub> H <sub>16</sub>	13.58
	5.23	β-Pinene	C <sub>10</sub> H <sub>16</sub>	42.26
	6.01	Eucalyptol	C <sub>10</sub> H <sub>18</sub> O	9.52
	7.59	Bicyclo [3.1.1] heptan-3-ol, 6,6-dimethyl-2-methylene-	C <sub>10</sub> H <sub>16</sub> O	3.86
	8.41	Octatriene, 1,3-trans-5-trans-	C <sub>8</sub> H <sub>12</sub>	3.93
	12.29	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S- (E, E)]-	C <sub>15</sub> H <sub>24</sub>	10.52
	17.33	2-Butanone, 3-methoxy-3-methyl-	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	3.14
	21.62	Bicyclo [5.2.0] nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	C <sub>15</sub> H <sub>24</sub>	13.19
	Clove	10.61	Eugenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
11.53		Caryophyllene	C <sub>15</sub> H <sub>24</sub>	10.40
12.72		Phenol, 2-methoxy-4-(2-propenyl)-, acetate	C <sub>12</sub> H <sub>14</sub> O <sub>3</sub>	17.15

negro pepper against *L. monocytogenes* except for Gram-negative bacteria which were not included in this study. Antimicrobial activity of clove and negro pepper is however evident based on their active compounds. Studies previously done attributed active compounds of clove such as eugenol (71.56%), thymol (0.87%), eugenyl acetate (8.99%), caryophenylene oxide (1.67%), nootkatin (1.05%), p-cymene (0.90), guaiol (0.90%), and isolongifolanone (0.86%) to antimicrobial activity (Nassar et al., 2007). The current study revealed three major chemical compounds of clove (Table 8), eugenol (72.45%), caryophyllene (10.45%), and phenol, 2-methoxy-4-(2-propenyl)-, acetate (17.15%). Interestingly, eugenol being the main bioactive component showed consistency with previously published literature. Similarly, Kusalaruk and Nakano (2021) studied the hurdle effects of plant extract against *E. coli* and reported similar chemical compositions of ethanolic clove extracts. Moreover, another study documented linalool, trans-β-ocimene, α-farnesene, α-pinene, β-pinene, camphene, and limonene in negro pepper as compounds contributing to antimicrobial activity (Tairu et al., 1999) against bacterial pathogens. Our study revealed eight bioactive components (Table 8) present in negro pepper which may have conferred inhibitory effects against the tested pathogens. These active compounds could penetrate the cell membrane and interact with key enzymes resulting in the loss of their functionality and thereby causing impairment of enzymatic mechanisms for energy production and metabolism. Similarly, many of these compounds interfere with phospholipid bilayer of the cell membrane and eventually lead to destabilization of plasma membrane, leakage of intracellular components, severe cell collapse and deformation, and inactivation of genetic materials (Ceruso et al., 2020; Takó et al., 2020).

Table 7 illustrates the MICs and MBCs of ethanolic spice extracts against *S. aureus*, MRSA, *B. cereus*, and *B. subtilis*. By comparison, the sensitivities of *S. aureus* and MRSA to the extracts were significantly higher than those of *B. cereus*, and *B. subtilis*. This could be ascribed to rapid morphological alterations resulting in significant number of pores, wrinkled surface, cell protrusions, and aggregation (Clemente et al., 2016; Efenberger-Szmechtyk et al., 2021) and also differences in virulent enzymatic response of *S. aureus* and MRSA to extracts compared to *B. cereus*, and *B. subtilis*. *Bacillus* species were least sensitive due to their resistance to environmental conditions and their ability to form resistant resting stages known as endospores (Liliwirianis et al., 2011). Polyphenolic compounds embedded in plant extracts are involved in multiple mode of action such as inhibiting protein and ATP synthesis and thereby causing changes in metabolic processes and inhibiting DNA synthesis (Efenberger-Szmechtyk et al., 2021) which could lead to cellular instability. Furthermore, it was noted that the bacteriostatic and bactericidal results of some test antimicrobial agents had MIC values equal to their MBCs. This explains the fact that these agents at such concentrations exert inhibitory effect against bacterial proliferation characterized by mortality induction.

In order to evaluate the killing kinetics of both negro pepper and clove, time-kill assays using inhibitory and bacteriostatic concentrations were performed. Time-kill kinetic study revealed that ethanolic fractions

of negro pepper and clove were effective against *L. monocytogenes*. As illustrated in Fig. 1, the extracts exerted strong activity against *L. monocytogenes* resulting in absolute mortality. Concentrations equal to 2xMIC and 4xMIC significantly reduced inoculum size by > 3 log<sub>10</sub> CFU/mL. Antibacterial agent was regarded listericidal at the lowest concentration that reduced the population size by > 3 log<sub>10</sub> CFU/mL (99.9%) and listeristatic, if the population decreased by 0–3 log<sub>10</sub> CFU/mL (Bag et al., 2012; Sharma et al., 2012).

## 5. Conclusions

This study revealed that the various plant extracts possess antibacterial activity. Only clove and negro pepper demonstrated potency against *L. monocytogenes*. Interestingly, negro pepper showed broad spectrum of activity eliciting bacteriostatic efficacy against all the tested pathogens. The present findings are promising information that support the antibacterial activity of the tested spices as potential candidates to combat bacterial pathogens associated with food-borne outbreaks. It could also encourage future research on the studies of synergism, activity in food or food processing systems, and mechanism of activity against specific pathogens. Furthermore, studies are needed to uncover pharmaceutical relevance of the tested plant materials and this report may serve as a steppingstone. To the best of our knowledge, data on the antibacterial efficacy of negro pepper against *L. monocytogenes* is reported here for the first time.

## CRedit authorship contribution statement

All authors have contributed enormously to this manuscript.

Christian Xedzro designed, planned, and performed the experiments, collected, and analysed the data, wrote the manuscript in consultation with Kwaku Tano-Debrah and Hiroyuki Nakano. Kwaku Tano-Debrah devised the project, the main conceptual ideas leading to this manuscript. This co-author suggested and assisted in getting the research samples. Hiroyuki Nakano worked out almost all the technical details, and assisted in relevant calculations for the experiment, supervised the project, revised the manuscript, gave technical assistance and provided research tools and equipment. All authors discussed the results, commented, and agreed on the manuscript.

## Funding

This research was supported and funded by the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) and Hiroshima University.

## Author statement

I would like to declare on behalf of my co-authors that this work has not been published previously, and not under consideration for publication elsewhere. No conflict of interest exists in the submission of this

manuscript.

## Declaration of Competing interest

Authors declare no conflicts of interest.

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