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ANTIBACTERIAL ACTIVITY OF CRUDE ETHANOLIC EXTRACTS AND ESSENTIAL OILS OF SPICES AGAINST SALMONELLAE AND OTHER ENTEROBACTERIA

Suree Nanasombat* and Pana Lohasupthawee

Department of Applied Biology, Faculty of Science,
King Mongkut's Institute of Technology Ladkrabang,
Chalongkrung Road, Ladkrabang, Bangkok 10520, Thailand

ABSTRACT

Crude ethanolic extracts and essential oils of 14 spices including cardamom, cinnamon, clove, coriander, cumin, garlic, ginger, holy basil, kaffir lime leaves and peels, lemongrass, mace, nutmeg, black and white pepper, and turmeric were examined for their antibacterial activity against 20 serotypes of *Salmonella* and 5 species of other enterobacteria using disk diffusion method as preliminary screening. Of these, 9 crude ethanolic extracts and 11 essential oils were selected to determine the minimum inhibitory concentrations (MICs) using microbroth dilution test. Among all ethanolic extracts, clove extract had the most inhibitory effect on the growth of all bacterial strains tested. Oils of clove and kaffir lime peels exhibited greater antibacterial activity against all tested strains, compared to other spice oils. The oils of cardamom, coriander, and cumin were also potent inhibitors of bacterial growth, showing the lowest MIC of 4.2 μl/ml to most bacterial strains tested. Both oil and ethanolic extract of kaffir lime peels showed greater antibacterial action, compared to the extracts of kaffir lime leaves. In general, inhibitory activity of spice oils was greater than that of their own ethanolic extracts. Of all serotypes of *Salmonella* tested, *Salmonella* Typhimurium (non-DT104 strain) is the most susceptible strain to both forms of spice extracts. On the other hand, *Salmonella* Derby and *Salmonella* Rissen were the most resistant strains to the extracts, followed by *Salmonella* Agona and *Salmonella* Typhimurium DT104. *Escherichia coli* was more susceptible to most of the spice oils than other non-salmonellae strains tested.

KEYWORDS: *Salmonella*, enterobacteria, spice extract, essential oil, antibacterial activity

1. INTRODUCTION

In recent years, food safety concerns have been focused on pathogens, such as *Salmonella* which is recognized as one of the leading causes of foodborne bacterial diseases. The problem of human salmonellosis following consumption of contaminated foods has increased worldwide. Based on reports from 1973 to 1997, cases of salmonellosis other than typhoid have been reported almost each year. These outbreaks were epidemiologically linked to the consumption of several types of foods including chocolate, egg drink, cuttlefish, mayonnaise, fruit soup, fresh fruits and vegetables, dairy products, and fermented meat products [1-4]. Fermented meat products including salami and Lebanon bologna have recently been linked with transmission of *Salmonella* [2, 5]. This bacterium has also been isolated from Thai food products, such as Thai fermented sausage, Thai fermented pork (nham), fermented fruits, papaya salad, fruit drink, ice cream, ground peanut, sugar-coated tamarind, and biscuit with filling [6-7]. This is of particular concern in food safety.

*Corresponding author. Tel: 66-2737-3000 ext. 6264 Fax: 66-2326-4414 E-mail: knsuree@kmitl.ac.th

The growing concern about safety of foods has recently led to the development of natural antimicrobials to control foodborne pathogens. Spices are some of the most commonly used natural antimicrobial agents in foods. Addition of spices in foods not only imparts flavor and pungent stimuli but also provides antimicrobial property [8-9]. Natural antimicrobial compounds in spices were found to possess antimicrobial activity [10-11]. Although some researchers have studied the antibacterial activity of spices against several species of bacteria, few serotypes of *Salmonella* have been tested, such as *S.* Typhimurium [11-14], *S.* Enteritidis [15], *S.* Infantis [16], and *S.* Anatum [17]. Antimicrobial activity of spices may differ between strains within the same species of bacteria. The sensitivity of each type of spices against several serotypes of *Salmonella* has not been reported. In addition, the antimicrobial property of spices may differ depending on the forms of spices added, such as fresh, dried, or extracted forms. In order to use spices to control *Salmonella* in foods, it is essential that antibacterial effects of crude ethanolic extracts and essential oils of spices against several serotypes of *Salmonella* be investigated. Therefore, the aim of this study is to determine antibacterial property of some spice extracts for future application as natural anti-*Salmonella* agents in Thai fermented meat products.

2. MATERIALS AND METHODS

2.1 Bacterial strains

Twenty five bacterial strains (20 serotypes of *Salmonella* and 5 species of other enterobacteria) were used in this study. *Salmonella* Agona (DMST 10338), *S.* Anatum (DMST 7108), *S.* Choleraesuis ssp. Choleraesuis (DMST 8014), *S.* Derby (DMST 8535), *S.* Enteritidis (DMST 10633), *S.* Lexington (DMST 4112), *S.* London (DMST 4110), *S.* Newport (DMST 7101), *S.* Rissen (DMST 7097), *S.* Senftenberg (DMST 7113), *S.* Virchow (DMST 10635), *S.* Weltevreden (DMST 10637), *S.* Typhimurium (DMST 0562, non-DT104 strain), *Citrobacter freundii* (DMST 1959), *Enterobacter aerogenes* (DMST 8841), *Escherichia coli* (DMST 4212), *Klebsiella pneumoniae* (DMST 8216), and *Serratia marcescens* (DMST 4228) were obtained from the culture collection of the Department of Medical Sciences, Ministry of Public Health, Thailand. An antibiotic-resistant strain of *Salmonella*, *S.* Typhimurium DT104 (8748A-1) was obtained from the Center of Food Safety, the University of Georgia, Griffin, USA. *S.* Panama (SAP 08904/02) was isolated from nham (Thai fermented pork), and the other five serotypes including *S.* Amsterdam (SAP 08913/02), *S.* Hardar (SAP 08907/02), *S.* Orion (SAP 08991/02), *S.* Schwarzengrund (SAP 08906/02), and *S.* Stanley (SAP 08956/02) were isolated from fresh pork [7]. Bacterial cultures were maintained on Nutrient Agar (NA)slopes (pH 6.8, Difco Laboratories). They were subcultured monthly and subsequently stored at 4°C.

2.2 Culture preparation

A loopful of 24 h surface growth on a NA slope of each bacterial strain was transferred individually to 5 ml of Brain Heart Infusion (BHI) broth (pH 7.6, Difco). After incubation at 37°C for 24 h, bacterial cells were collected by centrifugation at 3000 rpm for 15 min, washed twice and resuspended in 0.1% peptone water. Turbidity was adjusted to match that of a 5 McFarland standard (10^8 CFU/ml). Then, a 1:10 dilution of the cell suspension was performed to give an inoculum concentration of 10^7 CFU/ml.

2.3 Extractions of spices

Fourteen types of spices including cardamom, cinnamon, clove, coriander, cumin, garlic, ginger, holy basil, kaffir lime leaves and peels, lemongrass, mace, nutmeg, pepper (black and white), and turmeric were purchased at retail in Bangkok, Petchaburi, and Burirum, Thailand (Table 1). These spices were extracted by ethanolic extraction and steam distillation using the procedure as outlined below.

Table 1 List of spices and their edible parts

Spices	Botanical name	Plant parts
Cardamom	*Amomum krervanh* Pierre	Seeds
Cinnamon	*Cinnamomum verum* J.S.Presel	Barks
Clove	*Eugenia caryophyllus* (Sprengel) Bullock & Harrison	Flower buds
Coriander	*Coriandrum sativum*	Fruits
Cumin	*Cuminum cyminum* Linn.	Seeds
Garlic	*Allium sativum* Linn.	Bulbs
Ginger	*Zingiber officinale* Vern. Adrak	Rhizomes
Holy basil	*Ocimum sanctum* Linn.	Leaves
Kaffir lime	*Citrus hystrix* DC.	Leaves and Fruits
Lemongrass	*Cymbopogon citratus* (DC.) Stapf.	Rhizomes
Mace	*Myristica fragrans* Houtt.	Seed coat
Nutmeg	*Myristica fragrans* Houtt.	Fruits
Pepper, black and white	*Piper nigrum* Linn.	Fruits
Turmeric	*Curcuma longa* Linn.	Rhizomes

2.3.1 Preparation of crude ethanolic extracts

The spice materials were cut into small pieces; 20 g of each were soaked in 100 ml of 95% ethanol, and shaken at 150 rpm for 4 days at ambient temperature. The mixtures were then filtered. The filtrates were evaporated using vacuum rotary evaporator (BÜCHI Rotavapor R-200/205, Model R205V800), and frozen at -80°C before freeze drying (Labconco, Model Lyph. Lock 6). Stock solutions of crude ethanolic extracts were prepared by diluting the dried extracts with 10% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 400 mg/ml.

2.3.2 Preparation of essential oils

The small pieces of spice materials (300 g) were placed in a flask (2 L) together with distilled water (1 L). After steam distillation, the 100% pure essential oils were collected, dispensed into dark bottles, and stored at 4°C until used. The stock solutions of crude ethanolic extracts and essential oils were ready to use for disk diffusion test and determination of minimum inhibitory concentration.

2.4 Screening of spice extracts using disk diffusion technique

The disk diffusion test was performed using the standard procedure as described by Jorgensen *et al.* [18]. The inoculum suspension of each bacterial strain was swabbed on the entire surface of Mueller-Hinton agar (MHA, pH 7.3 ± 0.1, Difco). Sterile 6-mm filter paper discs (Schleicher & Schuell) were aseptically placed on MHA surfaces, and crude ethanolic extracts or essential oils were immediately added to discs in volumes of 20 µl or 15 µl, respectively. A 20-µl aliquot of 10% DMSO was also added to a sterile paper disc as a negative control, whereas a disc containing 10 µg amoxycillin was placed in the plate as a positive control. The plates were left at ambient temperature for 15 min to allow excess prediffusion of extracts prior to incubation at 37 °C for 24 h. Diameters of inhibition zones were measured. Each experiment was done in duplicate.

2.5 Determination of the minimum inhibitory concentration using microbroth dilution test

The dilution test was performed to determine minimum inhibitory concentrations (MICs) using the standard procedure as described by Jorgensen *et al.* [18]. One hundred microliters of Mueller-Hinton broth (MHB) were added in each well of a microtiter plate. The 100-µl aliquot of stock solution of crude ethanolic extract (400 mg/ml) was added, and subsequently two-

fold serially diluted with MHB. The inoculum suspenion (20 µl) of each bacterial strain was then added in each well containing crude ethanolic extract and MHB. The final concentrations of the extract were 166.7, 83.3, 41.7, 20.8, 10.4, 5.2, and 2.6 mg/ml. The MICs of essential oils were determined using a similar procedure. Different amounts of the oils (8, 6, 4, 2, 1, and 0.5 µl) were added to the broth cultures (120 µl) to get the final concentrations of 62.5, 47.6, 32.3, 16.4, 8.3, and 4.2 µl/ml. The negative and positive controls were also performed using 10% DMSO and penicillin G with the concentration of 50,000, 5,000, 500, 50, 5, 0.5, 0.05, and 0.005 unit/ml, respectively. Duplicate wells were run for each concentration of spice extracts. The plates were incubated at 37°C for 24 h, and the turbidity was measured at 620 nm using the microplate reader (iEMS Reader MF, Labsystems). The lowest concentration that inhibited visible growth of the tested organisms was recorded as the MIC.

3. RESULTS AND DISCUSSION

3.1 Preliminary screening of spice extracts

The results of the disk diffusion test indicated that crude ethanolic extracts of cardamom, cinnamon, clove, cumin, kaffir lime leaves, kaffir lime peels, and lemongrass showed different degrees of growth inhibition, depending on the bacterial strains (Table 2). The ethanolic extracts of clove, cumin, and kaffir lime peels showed the broadest antibacterial activity by inhibiting growth of all bacterial strains tested (the diameter of inhibition zone, 8-22 mm), while the extracts of cardamom, cinnamon, and kaffir lime leaves inhibited the growth of almost all strains (7-12 mm), except for *S.* Typhimurium, *S.* London, and *Serratia marcescens*. Lemongrass extract was active against only 17 strains (7-11 mm) of the 25 strains. Of all ethanolic extracts tested, the clove extract showed the highest antibacterial activity against some strains, such as *S.* Typhimurium (15 mm), and *S. marcescens* (22 mm). However, ethanolic extracts of coriander, garlic, ginger, holy basil, mace, nutmeg, black pepper, white pepper, and turmeric were inactive against all bacterial strains tested.

Oils of cardamom, clove, coriander, cumin, kaffir lime peels, and nutmeg inhibited the growth of all strains tested (8-21 mm), but those of garlic, ginger, holy basil, kaffir lime leaves, and mace inhibited the growth of only some strains (Table 3). Compared to other spice oils, the oils of clove and kaffir lime peels showed greater antibacterial activity against all strains tested, with zone diameters of 12-19 mm and 12-21 mm, respectively. Oils of cinnamon, lemongrass, black pepper, white pepper, and turmeric were not extracted because of very small amount of oils in plant materials.

In general, the inhibitory activity of essential oils was greater than that of ethanolic extracts, especially clove and kaffir lime peels. Among the serotypes of *Salmonella* tested, the ethanolic extracts and oils exhibited slightly different degree of inhibition. *S.* Typhimurium (non-DT104 strain) was the most susceptible serotype to both oils and extracts of clove and kaffir lime's peels. Of all enterobacteria tested, *S. marcescens* was the most susceptible strain to both forms of clove extracts.

Table 2 Antibacterial activity of crude ethanolic extracts of spices against *Salmonella* spp. and other enterobacteria using disk diffusion test

Crude ethanolic extracts	Diameter of Inhibition Zone (mm)[a]																								
	S. Agona	*S.* Amsterdam	*S.* Anatum	*S.* Choleraesuis	*S.* Derby	*S.* Enteritidis	*S.* Hadar	*S.* Lexington	*S.* London	*S.* Newport	*S.* Orion	*S.* Panama	*S.* Rissen	*S.* Senftenberg	*S.* Schwarzengrund	*S.* Stanley	*S.* Virchow	*S.* Weltevreden	*S.* Typhimurium (non-DT104 strain)	*S.* Typhimurium DT104	*Citrobacter freundii*	*Enterobacter aerogenes*	*Escherichia coli*	*Klebsiella pneumoniae*	*Serratia marcescens*
Cardamom	10	9	7	11	9	10	10	10	10	10	9	9	10	9	8	10	10	10	-	9	12	10	10	10	10
Cinnamon	9	7	9	9	9	10	8	8	-	9	8	8	8	10	7	8	9	10	8	9	9	8	8	8	9
Clove	10	9	11	11	10	10	10	12	11	11	11	10	10	11	9	10	11	11	15	10	12	10	10	10	22
Coriander	-[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cumin	9	9	11	11	10	10	10	9	10	10	9	9	10	11	9	9	10	11	10	8	9	10	10	10	9
Garlic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ginger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Holy basil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kaffir lime leaves	10	10	10	10	10	10	7	8	8	10	10	9	10	9	9	9	11	10	10	10	8	10	9	8	-
Kaffir lime peels	10	10	10	9	11	10	9	10	10	10	10	10	10	11	10	9	10	9	11	8	10	10	11	9	10
Lemongrass	-	10	10	10	10	10	-	10	10	10	-	-	10	10	-	10	11	10	11	10	-	-	7	7	-
Mace	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nutmeg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pepper,Black	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pepper,White	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turmeric	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amoxycillin	26	22	27	26	-	22	21	23	23	27	-	-	20	8	-	-	25	25	29	-	16	-	17	-	12
10%DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a]Data are mean of two replications.
[b]No inhibition was observed.

Table 3 Antibacterial activity of essential oils of spices against *Salmonella* spp. and other enterobacteria using disk diffusion test

Essential oils	Diameter of Inhibition Zone (mm)[a]																							
	S. Agona	*S.* Amsterdam	*S.* Anatum	*S.* Choleraesuis	*S.* Derby	*S.* Enteritidis	*S.* Hadar	*S.* Lexington	*S.* London	*S.* Newport	*S.* Orion	*S.* Panama	*S.* Rissen	*S.* Senftenberg	*S.* Schwarzengrund	*S.* Stanley	*S.* Virchow	*S.* Weltevreden	*S.* Typhimurium (non - DT104 strain)	*S.* Typhimurium DT104	*Citrobacter freundii*	*Enterobacter aerogenes*	*Escherichia coli*	*Klebsiella pneumoniae*	*Serratia marcescens*
Cardamom	10	9	11	10	10	10	10	9	11	10	10	10	10	10	10	10	10	10	14	9	10	10	11	11	11
Clove	15	12	15	15	13	13	15	15	15	15	13	13	12	15	12	12	15	14	16	11	15	13	14	13	19
Coriander	10	10	11	11	9	8	10	9	10	10	11	10	9	10	10	9	10	10	12	9	10	10	10	12	12
Cumin	10	9	8	10	9	8	11	8	10	9	10	9	9	10	10	9	9	8	10	9	12	9	11	13	13
Garlic	8	8	9	9	8	8	9	8	8	8	8	9	8	8	8	9	9	9	10	8	8	8	10	8	-
Ginger	7	-	8	8	8	8	8	-	-	8	8	-	8	8	-	-	7	-	10	8	8	-	9	10	12
Holy basil	-[b]	-	9	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	-	-	-	9	10	8
Kaffir lime leaves	7	9	8	8	8	8	8	8	7	-	-	7	-	8	7	7	9	8	10	8	8	-	8	10	9
Kaffir lime peels	15	14	15	16	15	15	14	15	15	16	16	15	12	15	15	16	15	14	21	14	16	14	16	16	15
Mace	-	-	9	-	8	-	9	-	9	8	-	-	-	7	-	-	9	7	9	7	9	-	-	7	10
Nutmeg	9	9	9	9	9	9	9	10	9	10	9	9	9	9	9	10	10	9	10	8	10	10	10	10	10
Amoxycillin	26	22	27	26	-	22	21	23	23	27	-	-	20	8	-	-	25	25	29	-	16	-	17	-	12
10%DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a]Data are mean of two replications.
[b]No inhibition was observed.

3.2 Determination of minimum inhibitory concentration

Nine serotypes of *Salmonella* including *S.* Agona, *S.* Anatum, *S.* Choleraesuis, *S.* Derby, *S.* Enteritidis, *S.* Rissen, *S.* Senftenberg, *S.* Typhimurium (non-DT104 strain), and *S.* Typhimurium DT104 which are potential pathogens and most commonly isolated from fresh and fermented meat, and four species of other enterobacteria including *C. freundii*, *E. aerogenes*, *E. coli*, and *K. pneumoniae* were selected as test organisms for the MIC determination of spice extracts. The MIC values of crude ethanolic extracts of cardamom, cinnamon, clove, cumin, garlic, holy basil, kaffir lime leaves, kaffir lime peels, and lemongrass indicated that clove had the highest antibacterial action against 13 bacterial strains tested, followed by kaffir lime peels and holy basil (Table 4). Of all serotypes of *Salmonella* tested, *S.* Typhimurium (non-DT104 strain) was the most susceptible strain to most of the ethanolic extracts, while *S.* Derby was the most resistant bacterium, followed by *S.* Rissen, *S.* Agona, and *S.* Typhimurium DT104. *E. aerogenes* and *E. coli* were also resistant to most of the ethanolic extracts. Similarly, *S.* Typhimurium (non-DT104 strain) was also the most sensitive to Penicillin G (the MIC of 0.5 unit/ml), whereas *S.* Derby, *S.* Typhimurium DT104, and *E. aerogenes* were the most resistant (MIC of 5,000 unit/ml).

Oils of cardamom, clove, coriander, cumin, and kaffir lime peels strongly inhibited the growth of almost all strains tested (MIC of 4.2 µl/ml), except for *S.* Rissen which required higher MIC (Table 5). The oils of clove and cumin had the lowest MIC (4.2 µl/ml) to inhibit the growth of every strain tested. The oils of cardamom, coriander, and kaffir lime peels were also highly inhibitory. Nutmeg and mace oils exhibited slightly different degrees of inhibition. They inhibited the growth of most bacterial strains with the same MIC value, except for *S.* Typhimurium (non-DT104 strain), *E. aerogenes*, and *K. pneumonia*. The high MIC (>62.5 µl/ml) of kaffir lime leave and holy basil oils was needed to inhibit the growth of almost all bacterial strains tested, except for *S.* Typhimurium (4.2 µl/ml). Both oil and ethanolic extract of kaffir lime peels inhibited the bacterial growth more efficiently than those of kaffir lime leaves did. The MIC values of garlic and ginger oils varied depending on the bacterial strains. Ginger oil seemed to be a more potent inhibitor to most bacterial strains than garlic oil. While ginger oil showed great inhibitory effect (4.2 µl/ml) to *S.* Choleraesuis, *S.* Senftenberg and *E. coli*, garlic oil exhibited less inhibitory activity (16.4 µl/ml) against *S.* Typhimurium. Of all serotypes of *Salmonella* tested, *S.* Typhimurium (non-DT104 strain) was the most susceptible to most of the spice oils, while *S.* Rissen was the most resistant, followed by *S.* Derby, *S.* Agona, *S.* Typhimurium DT104, and *S.* Senftenberg. Among the non-salmonellae strains tested, *E. coli* was the most susceptible strain to most of the spice oils.

In this study, the crude ethanolic extracts and essential oils of 14 spices were screened for their antibacterial properties. The degree of antibacterial activity was considered from the MIC values against the bacterial strains. Oka [19] demonstrated that the adsorption of spice preservative on the bacterial cell depended on its concentration. The results of the present study indicated that clove exhibited the strongest antibacterial activity in both forms of extracts, followed by kaffir lime peels. The antibacterial activity of clove is attributed to eugenol (2-methoxy-4-allyl phenol). Clove bud oil contains high eugenol (70-90%) content [20]. This compound is an antimicrobial compound having wide spectra of antimicrobial effect [11, 21] which may contribute to growth inhibition of enterobacteria. High tannin content (10-19%) in clove provides additional antimicrobial activity [10]. Similar findings have been reported by other researchers [22-23].

Table 4 Minimum inhibitory concentrations of crude ethanolic extracts of spices against *Salmonella* spp. and other enterobacteria

Crude ethanolic extracts	Minimum inhibitory concentrations (mg/ml)													
	S. Agona	*S.* Anatum	*S.* Choleraesuis	*S.* Derby	*S.* Enteritidis	*S.* Rissen	*S.* Senftenberg	*S.* Typhimurium (non –DT 104 strain)	*S.* Typhimurium DT104	*Citrobacter freundii*	*Enterobacter aerogenes*	*Escherichia coli*	*Klebsiella pneumoniae*
Cardamom	83.3	83.3	41.7	>166.7	>166.7	>166.7	>166.7	20.8	83.3	41.7	>166.7	83.3	41.7
Cinnamon	166.7	83.3	166.7	166.7	166.7	>166.7	83.3	166.7	166.7	41.7	166.7	>166.7	83.3
Clove	5.2	2.6	2.6	20.8	2.6	5.2	5.2	2.6	2.6	10.4	10.4	2.6	41.7
Cumin	166.7	>166.7	41.7	>166.7	83.3	166.7	166.7	>166.7	166.7	>166.7	166.7	>166.7	41.7
Garlic	166.7	83.3	83.3	>166.7	83.3	166.7	83.3	41.7	83.3	83.3	>166.7	83.3	>166.7
Holy basil	41.7	20.8	41.7	83.3	83.3	41.7	83.3	41.7	41.7	41.7	83.3	83.3	10.4
Kaffir Lime leaves	166.7	>166.7	166.7	>166.7	166.7	166.7	>166.7	83.3	166.7	166.7	>166.7	166.7	5.2
Kaffir Lime peels	41.7	83.3	41.7	83.3	41.7	41.7	41.7	41.7	41.7	41.7	83.3	41.7	>166.7
Lemongrass	166.7	166.7	83.3	166.7	166.7	166.7	166.7	83.3	166.7	166.7	>166.7	166.7	>166.7
Positive control (unit/ml)													
Penicillin G	50	50	50	5,000	500	50	50	0.5	5,000	500	5,000	0.5	50

Table 5 Minimum inhibitory concentrations of essential oils of spices against *Salmonella* spp. and other enterobacteria

Essential oils	Minimum inhibitory concentrations (µl/ml)													
	S. Agona	*S.* Anatum	*S.* Choleraesuis	*S.* Derby	*S.* Enteritidis	*S.* Rissen	*S.* Senftenberg	*S.* Typhimurium (non – DT 104 strain)	*S.* Typhimurium DT104	*Citrobacter freundii*	*Enterobacter aerogenes*	*Escherichia coli*	*Klebsiella pneumoniae*
Cardamom	4.2	4.2	4.2	4.2	4.2	8.3	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Clove	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Coriander	4.2	4.2	4.2	4.2	4.2	>62.5	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Cumin	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Garlic	62.5	47.6	47.6	>62.5	47.6	>62.5	47.6	16.4	62.5	>62.5	>62.5	47.6	62.5
Ginger	8.3	8.3	4.2	>62.5	>62.5	8.3	4.2	8.3	>62.5	>62.5	>62.5	4.2	>62.5
Holy basil	>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	4.2	>62.5	>62.5	>62.5	>62.5	>62.5
Kaffir lime leaves	>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	4.2	>62.5	>62.5	>62.5	>62.5	>62.5
Kaffir lime peels	4.2	4.2	4.2	4.2	4.2	8.3	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Mace	8.3	4.2	4.2	8.3	4.2	>62.5	8.3	8.3	4.2	4.2	47.6	4.2	8.3
Nutmeg	8.3	4.2	4.2	8.3	4.2	>62.5	8.3	4.2	4.2	4.2	4.2	4.2	4.2
Positive control (unit/ml)													
Penicillin G	50	50	50	5,000	500	50	50	0.5	5,000	500	5,000	0.5	50

Kaffir lime peels also contain antimicrobial compounds. The major constituents in essential oil of kaffir lime peels are β-pinene (30.6%), limonene (29.2%), sabinene (22.6%), whereas the main compound in kaffir lime leaves is citronellal (65.4%) [24]. β-pinene and limonene had greater inhibitory activity against *S.* Enteritidis than citronellal [8]. Uribe *et al.* [25] reported that β-pinene inhibited the respiration of both intact cell of *Saccharomyces cerevisiae* and its isolated mitochondria. This may be the reason why kaffir lime peels showed greater antibacterial activity than kaffir lime leaves.

The oils of cumin, cardamom, and coriander were also highly inhibitory to the tested bacteria so they may contain potent antimicrobial compounds. The major constituents of these oils are: cuminaldehyde (20-72%) and monoterpene hydrocarbons (e.g. β-pinene, γ-terpinene, p-cymene) in cumin oil; 1,8-cineole (20-60%) and α-terpinyl acetate (20-53%) in cardamom; linalool (74%) and other components (small amounts of α-pinene, γ-terpinene, geranyl acetate, camphor and geraniol) in coriander oil [20, 26]. Mace and nutmeg oils moderately inhibited the tested bacteria, with similar degree of antibacterial action. Nutmeg oil is much resembling mace oil. Nutmeg oil contains monoterpene hydrocarbons (61-88%, e.g. α-pinene, β-pinene, sabinene), oxygenated monoterpenes (5-15%), and aromatic ethers (2-18%, e.g. myristicin, elemicin, saffrole) [20], whereas mace oil consists of monoterpenes (87.5%), monoterpene alcohols (5.5%), and other aromatics (7.0%) [27]. Garlic and ginger oils possessed moderate antibacterial activity in this study. *E. coli* was more sensitive to garlic extracts than *E. aerogenes* which is in agreement with previous observations [28]. The major antimicrobial compound in garlic is allicin [29]. Garlic extracts have been found to possess antibacterial property against several bacteria including *S.* Typhimurium, *S.* Typhi, *E. coli*, *Bacillus cereus*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* [28, 30-31]. The major pungent components of ginger are gingerone and gingerol which have strong inhibitory activity against pathogenic bacteria [8].

In the present study, most of the spice oils exhibited stronger antibacterial activity than their own ethanolic extracts, with the exception of holy basil. The antimicrobial property of spices has been shown to be attributable to the essential oil fraction [32]. This is because of the fact that some essential oils contain active components which influence certain metabolic functions of microbial cells. As most components of spice oils belong to the terpenoid family, there has been much speculation on the contribution of the terpene fraction of the oils to their antimicrobial activity [29]. Some researchers have demonstated the antimicrobial activity of the most common terpene compounds, such as thymol, carvacrol, linalool, eugenol, α-pinene, and β-pinene in spices against several microbial strains [8, 11-12]. Cyclic terpene compounds have been reported to cause loss of membrane integrity and dissipation of proton motive force [33]. Wilkins and Board [34] suggested that the antimicrobial action of spices is due to the impairment of a variety of enzyme systems involving in the production of energy or synthesis of structural components in microbial cells.

4. CONCLUSIONS

In conclusion, the degree of antibacterial property of spices tested can be put in the following order: clove > kaffir lime peels > cumin > cardamom > coriander > nutmeg > mace > ginger > garlic > holy basil > kaffir lime leaves. These spices may be selected for use as potentially useful anti-*Salmonella* agents in fermented meat products and other foods, depending upon the desired flavor of the products. The oil fraction of these spices is recommended, with the exception of holy basil which should be used in the form of ethanolic extract. Of all serotypes of *Salmonella* tested, *S.* Typhimurium (non-DT 104 strain) is the most vulnerable to crude ethanolic extracts and oils of spices, while *S.* Derby and *S.* Rissen were the most resistant. *E. coli* was more sensitive to most of the spice oils than other non-salmonellae strains tested. However, there are some limitations in using spices, such as 1) the decreasing of antimicrobial activity when spices are added to food materials containing protein, carbohydrate, and fat, and 2) the strong flavor of some spices. The overall flavor of the products may not be acceptable if

a large amount of spices need to be added in the products in order to inhibit the pathogenic bacteria. A possible way is to use spices in combination with other preservatives such as acid, salt, sugar, and other chemical preservatives, or other food preservation systems such as thermal processing, freezing, cold storage, etc. [10, 35].

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