International Food Research Journal 26(3): 933-944 (June 2019)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antibacterial activity of ethanolic *Piper cubeba* L. extract against *Escherichia coli* and its effect on microbiological quality of raw chicken meat during storage

[1]Fazly Ann, Z. and [1,2*]Rukayadi, Y.

[1]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
[2]Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

Article history

Received: 25 July, 2018
Received in revised form: 3 January, 2019
Accepted: 12 February, 2019

Keywords

Escherichia coli
Chicken meat
Microbial safety
Piper cubeba L.
Storagey

Abstract

Piper cubeba L. is traditionally recognised as flavouring ingredient in various types of foods and has been used to marinate meat. Scientifically, it has been reported to possess various valuable nutritional and pharmacological properties including antimicrobial potential. The aim of the present work was to determine the antibacterial activity of ethanolic *P. cubeba* L. extract against *Escherichia coli* and its effect on the microbiological quality of raw chicken meat during storage. Disc diffusion assay was done and resulted in 8.40 ± 0.10 mm of inhibition zone. The bacteriostatic and bactericidal effects of the extract were determined at 0.63 ± 0.00 mg/mL and 1.25 ± 0.00 mg/mL of concentration by MIC and MBC methods, respectively. The killing time was recorded at 2 × MIC (1.25 mg/mL) for 4 h. The application of the extract on chicken meat samples showed reduction in TPC and *E. coli* count with the observed optimum condition at 5.00% concentration stored at -18°C for 14 days based on the consistent reduction. Sensory attributes acceptability evaluation by 9-point hedonic scale showed acceptable score for colour, odour, texture and overall acceptability of the treated raw chicken meat samples. The findings implies that *P. cubeba* L. can be listed as one of the alternatives to reduce the bacterial load of raw chicken meat prior to cooking which is very important in ensuring food safety as well as reducing the occurrence of foodborne poisoning associated with chicken meat..

Introduction

Piper cubeba L., also known as '*kemungkus*' in Malaysia, is the most valuable spices in Europe since the medieval period. The plant belongs to the Piperaceae family and Piper genus. It is recognised as an important source of pepper in the global spice market. This climbing shrub plant originated from Indonesia and mostly grown in Java and Sumatera (Nahak and Sahu, 2011). Traditionally, *P. cubeba* L. have been used to marinate and season the meat. It was also used in traditional medicine for the treatment of fever, gastroenteritis, dental diseases, gonorrhoea, syphilis and asthma (Mouid *et al*., 2016). In Malaysia, the pepper has been used in treating rheumatism, while in China it was used in traditional medicine due to its warming properties (Lim, 2012). Scientifically, researchers have reported various nutritional and pharmacological properties of the pepper. Nutritionally, it is a good source of essential elements such as potassium and iron (Fatima *et al*., 2012). The pepper contains beneficial phytochemicals such as amides, alkaloids (piperine and cubebin), flavones, flavanones, lignans, neolignans, sesquiterpenes, terpenes and propenylphenols (Parmar *et al*., 1997). The pharmacological properties of the pepper include anti-inflammatory, antimicrobial, anti-oxidant, anti-carcinogen and anti-ulcer. The metabolites and derivatives of the plant exhibit bacteriostatic and bactericidal effects through various mechanisms including inhibiting the bacterial growth, breaking down the cell membrane and attaching to the bacterial DNA (Al-Tememy, 2013).

Malaysian poultry production accounts for 90% in Peninsular Malaysia and the rest in East Malaysia (Poultry World, 2014). According to USDA (2015a), Malaysia is self-sufficient in poultry meat production and about 40% of the meats are marketed to the consumers through wet market. Chicken meat is considered as the main source of protein in Malaysian

*Corresponding author.
Email: yaya_rukayadi@upm.edu.my

diet across all the ethnic groups. It is the most popular meat among other poultry species (Shafini *et al*., 2017). According to the Department of Veterinary Services (DVS, 2017), per capita consumption of poultry meat in 2016 was 50.32 kg, which was the highest among other livestock products. The meat is perishable and highly susceptible to microbial contamination where more than 30 genera of microorganisms including foodborne pathogens have been reported contaminating the meat. The bacteriological analysis from various studies have shown the presence of *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Campylobacter* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, *Clostridium perfringens*, *Arcobacter* spp. and *Helicobacter* spp. on the raw chicken meat samples (Mead, 2004; Kozačinski *et al*., 2006; Adu-Gyamfi *et al*., 2012). Of the listed bacteria, CDC (2017) classified five as foodborne pathogens; *E. coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens* and *Campylobacter* spp., with *Salmonella* spp. and *E. coli* being identified as the two most important foodborne pathogens of public health interest incriminated in chicken meat globally (Adeyanju and Ishola, 2014).

Contamination of chicken meat with the pathogens that may lead to foodborne poisoning is an important public health issue. In the cases of food poisoning due to *E. coli* O157:H7 associated with chicken meat products, symptom such as acute haemorrhagic diarrhoea may develop (Chang *et al*., 2013). Microbiological contamination may occur at any point of supply chain such as during slaughtering, post-slaughter handling, food preparation, transportation and storage (Shafini *et al*., 2017). Consumption of cross-contaminated or undercooked chicken meat with high bacterial load will potentially risk the consumer. Seeing as *P. cubeba* L. has abundant nutritional and pharmacological properties, the use of its extract on chicken meat is believed to reduce the bacterial load prior to cooking. Therefore, the present work was conducted to determine the antibacterial activity of *P. cubeba* L. extract against *E. coli* and to identify the optimum concentration, exposure time and storage temperature.

Materials and methods

Sample collection

Dried *P. cubeba* L. berries were purchased at herbal traditional market, Pasar Baru, Bandung, Indonesia. The samples were kept in seal plastic bags at room temperature before further processing. The chicken meat samples from wet market (GPS coordinate: 2.984616, 101.669595) and supermarket (GPS coordinate: 2.983479, 101.662725) were purchased at Seri Kembangan, Selangor and stored in the ice box at 4 ± 2°C during transportation. The samples were immediately processed in less than 1 h upon arrival at Food Microbiology Laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia, Selangor.

Extraction of Piper cubeba L.

The extraction of *P. cubeba* L. was conducted following the method described by Rukayadi *et al*. (2008) with slight modification. Briefly, 300 g dried berries were grinded using a dry blender Panasonic MX-GM1011 (Panasonic Corporation, Osaka, Japan). The grinded berries were then soaked in 99.8% ethanol (Sigma-Aldrich, Missouri, USA) with the ratio of 1:10 for 7 d at room temperature and occasionally shaken every alternate day. At the end of 7 d, the soaked berries were vacuum-filtered through Whatman filter paper No. 2 (Whatman International Ltd, Middlesex, England) by EYELA aspirator pump (Tokyo Rikakikai Co., Tokyo, Japan). The filtrate was then concentrated by using a rotary vacuum evaporator Heidolph VV2011 (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 50°C and 150 rpm for 30 to 40 min. The temperature was increased up to 85°C for two times for 30 s during the concentration process to obtain ethanol free extract. The crude extract was then stored at 4°C prior to use.

Preparation of Piper cubeba L. extract for treatment

Ethanolic *P. cubeba* L. extract was made by adding 1.0 mL extract into 99.0 mL 10% dimethylsulfoxide (DMSO) (R & M Marketing, Essex, UK) to a final concentration of 1.00% for the use in antibacterial activity determination of *E. coli* by disc diffusion assay (DDA), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and time-kill curve assay. For bacterial enumeration of chicken meat samples, the extracts were prepared at 0.00%, 0.05%, 0.50% and 5.00%. The 5.00% concentration was made by adding 5.0 mL extract into 95.0 mL 10% DMSO. 0.50% and 0.05% concentrations were prepared by addition of 99.50 mL and 99.95 mL 10% DMSO into 0.50 mL and 0.05 mL extract, respectively. The 10% DMSO was used as it was found not to kill most microorganisms (Yusoff *et al*., 2015). Sterile deionised water (DIW) (B. Braun Medical Industries, Penang, Malaysia) was used as 0.00% ethanolic *P. cubeba* L. extract concentration.

Disc diffusion assay (DDA)

Disk diffusion assay was performed following the technique described by CLSI (2013). A single colony of freshly prepared inoculum of *E. coli* O157:H7 (ATCC 43895) obtained from American Type Culture Collection (Maryland, United States) was spread on Müller-Hinton agar (MHA) by using sterile cotton swab. Sterile paper disks (6 mm diameter) (Schleicher and Schuell, Dassel, Germany) were place on the agar and infused with 20 µL 1.00% of *P. cubeba* L. extract for treatment, 1.00% of chlorhexidine (CHX) (Sigma, St. Louis, MO, USA) for positive control, and 10% DMSO (R & M Marketing, Essex, UK) for negative control. The plates were then incubated at 37°C for 24 h.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) of the extract was determined by broth microdilution method using sterile 96-wells round bottom microtiter plate (Greiner, Germany) with inoculum suspension of *E. coli* was adjusted from 10^6 to 108 CFU/mL. Column 1 of the well was filled with 200 µL Müller-Hinton broth (MHB) which served as negative control. Column 2 served as positive control, which was filled with medium and 200 µL bacterial suspension. The microdilution was performed at extract concentrations ranging from 5.000 mg/mL in column 12 to 0.009 mg/mL in column 3. The plate was incubated for 24 h at 37°C. The minimum bactericidal concentration (MBC) was then determined by sub-culturing 10 µL suspension from each MIC wells onto MHA plate, and incubated at 37°C for 24 h to evaluate the mean value of MIC and MBC.

Determination of time-kill curve

Time-kill assay was performed on the *E. coli* O157:H7 (ATCC 43895) by using ethanolic extracts of *P. cubeba* L. following the method described by CLSI (2012), with slight modification. The extract at 0.0 × MIC, 0.5 × MIC, 1.0 × MIC, 2.0 × MIC and 4.0 × MIC were prepared and added to MHB containing approximately 10^6 CFU/mL of inoculum suspension to a final volume of 1 mL. The mixture was then incubated at 37°C with 200 rpm agitation. At 0.0, 0.5, 1.0, 2.0, and 4.0 h, 100 µL of the each samples were serially diluted and plated onto MHA by using spread plate technique. The plates were then incubated at 37°C for 24 h prior to enumeration. The total plate count (TPC) was recorded and the graph of $\log_{10}$ CFU/mL versus time was plotted.

Bacterial load enumeration of untreated and treated chicken meat samples

The chicken meat samples were cut into 2.0 × 2.0 × 1.5 cm with an average of 3 ± 1 g per sample. Each of the samples from wet market and supermarket were then homogenised in BAGLIGHT BagSysytem stomacher bag (Interscience, Saint-Nom-la-Breteche, France) using stomacher machine BagMixer 400-P (Interscience, Saint-Nom-la-Breteche, France) for 2 min. Serial dilution from 10^{-1} to 10^{-8} were performed by the addition of 1 mL from each samples series into 9 mL 1% bacteriological peptone water solution (Oxoid Ltd, Hampshire, UK). For TPC, 0.05 mL of each dilution was pipetted into Petri dishes with Plate Count agar (PCA) and incubated at 37°C for 24 h. The visible colonies formed in the agar were counted and calculated in logarithm numbers of colony forming unit per grams ($\log_{10}$ CFU/g) of samples. For *E. coli* count, 0.05 mL of each dilution was pipetted into petri dishes with Eosin Methylene Blue (EMB) agar. Log_{10} CFU/g of samples was calculated by observation and enumeration of the presumptive colonies (green-metallic sheen colonies) that formed after incubation at 37°C for 24 h.

The raw chicken meat cuts were soaked in filtered tap water and four different concentrations of *P. cubeba* L. extract (0.00%, 0.05%, 0.50% and 5.00%) at two different exposure times of 5 and 10 min. The treated samples were individually kept in sterile universal bottles. All the samples were then stored at two different temperatures of -18 ± 2°C and 4 ± 2°C for 14 d. During storage, the TPC and *E. coli* count for treated chicken meat samples were calculated at 0, 7 and 14 days. The frozen stored samples post 7 and 14 days were thawed at 4 ± 2°C for 8 h prior to analysis. The respective treated samples were then homogenised using stomacher for 2 min, and serial dilution from 10^{-1} to 10^{-5} were performed prior to culturing on PCA for TPC and EMB agar for *E. coli* count. The means value of bacterial populations from each treatment series were calculated in duplicate for each experiment.

Sensory evaluation

The test was performed for each of the treatments of raw chicken meat samples following Brasil *et al*. (2012), with slight modification. The samples were treated with filter tap water, 0.00%, 0.05%, 0.50% and 5.00% at 5 and 10 min exposure times. A group consisting of 30 random untrained panellists were invited to evaluate each of the treatment samples presented with ten different 3-digit coded samples placed in a random order. The evaluation was conducted based on 9-point hedonic scale for

acceptance in terms of colour, odour, texture and overall acceptability. The rating for each sample ranged from extremely dislike (scale of 1) to extremely like (scale of 9) as described by Poste *et al*. (1991).

Statistical analysis

Minitab® Statistical Software (Minitab 16) (Minitab Inc., Pennsylvania, USA) was used to perform statistical analysis. MIC and MBC data were analysed by applying two samples T-test with significant difference determined at 99% confidence level ($p < 0.01$) between the treatment and positive control. For bacterial enumeration of untreated chicken meat samples, two sample T-test was performed with significant difference determined at 99% confidence level ($p < 0.01$) between treatment groups. Bacterial enumeration in treated chicken meat samples, the general linear model (GLM) and variance (One-Way ANOVA) with post-hoc Tukey's test were used to determine the significant effect at 95% confidence level ($p < 0.05$) between those treatment groups. Sensory attributes acceptability for individual score from each treatment was calculated and analysed using One-Way ANOVA with post-hoc Tukey's test to determine the significance effect at 95% confidence level ($p < 0.05$).

Results and discussions

P. cubeba L. extraction yield

The grinded *P. cubeba* L. berries extracted using ethanol by maceration method resulted in slightly viscous crude oil appearing in blackish brown colour with 18.0 ± 1.0% recovery yield. The ratio of 1:10 (w/v) for dry weight sample to solvent was used as an ideal recommendation by Pandey and Tripathi (2014). According to Lawson *et al*. (2010), the duration of solvent-sample soaking affects the efficiency of extraction with longer duration results in higher extraction yield. In the present work, the grinded berries were soaked in 99.8% ethanol for 7 d. The soaking processed was intended to break the cell wall of the plants and release the soluble phytoconstituent. Short period of soaking will have less sufficient time for the solvent to recirculate the extract of total available oil in plant extract compounds (Efthymiopoulos *et al*., 2019).

Disc diffusion assay (DDA)

The determination of antibacterial activity of ethanolic *P. cubeba* L. extract against *E. coli* O157:H7 (ATCC 43895) by DDA showed 8.40 ± 0.10 mm inhibition zone. Based on the principle, larger inhibition zone shows the greater antibacterial activity of the extract. In comparison with the zone (11.90 ± 0.50 mm) created by chlorhexidine (CHX) which served as positive control, even though the inhibition zone of the extract was smaller, the developed zone indicated the antibacterial activities of the extract against the tested *E. coli*. The same method has also been done by Al-Tememy (2013) which resulted in 10.00 mm inhibition zone for *E. coli*, 13.00 mm for Pseudomonas aeruginosa and 15.00 mm for *Staphylococcus aureus*, by using 100 µL ethanolic *P. cubeba* L. extract on the tested disc. Another study by Al Rdheha Ali *et al*. (2016) showed the antibacterial activity of 10%, 20% and 40% ethanolic *P. cubeba* L. extracts at 8.30 mm, 13.00 mm and 13.00 mm inhibition zone, respectively. The study also found that 20% hot aqueous extract resulted in the best inhibition against the tested *E. coli* at 20.00 mm, followed by cold aqueous at 15.00 mm and ethanolic at 12.00 mm.

MIC and MBC values of P. cubeba L. extract

MIC is considered as the gold standard in determining the susceptibility of microorganisms towards antimicrobials (Andrews, 2001). It is defined as the lowest concentration or the highest dilution of the antimicrobial agent that visually inhibits the growth of microorganisms after overnight incubation (Talaro and Chess, 2012). The lowest concentration of antimicrobial agent that did not show any growth of microorganisms was considered as the MBC (Aamer *et al*., 2014). In the present work, the MIC and MBC values of ethanolic *P. cubeba* L. extracts against *E. coli* O157:H7 (ATCC 43895) were determined, and the results for both test are presented as in Table 1. Based on the results, even though *P. cubeba* L. extract required higher concentration (0.63 ± 0.00 mg/mL) to inhibit the growth of the bacteria, and lower concentration (1.25 ± 0.00 mg/mL) to express the bactericidal effect as compared to the positive control, statistically both MIC and MBC values of extract and the control showed no significant different. The findings suggested that the antibacterial effect of *P. cubeba* L. extract used in the present work was performing as effectively as the commercial antibiotic towards the tested *E. coli*.

Study on MIC values of *P. cubeba* L. extract using acetone, chloroform, ethanol and aqueous as solvents against *E. coli* also has been done previously and resulted in 0.50, 0.50, 1.00 and 1.00 mg/mL concentrations, respectively (Al-Tememy, 2013). Another study by Rukayadi *et al*. (2013) gave the MIC and MBC values for methanolic *P. cubeba* L. extract against *E. coli* (ATCC 25922) at

3.20 and 6.40 mg/mL, respectively. In comparison with the ethanolic extract of other herbs and spices, the MIC and MBC against *E. coli* (ATCC 11303) were identified at higher values such as 10.00 mg/mL (MIC), 10.00 mg/mL (MBC) for *Origanum vulgare* L. (oregano), 10.00 mg/mL (MIC), 20.00 mg/mL (MBC) for *Salvia officinalis* L. (sage), 10.00 mg/mL (MIC), 10.00 mg/mL (MBC) for *Syzygium aromaticum* (L.) Merrill & Perry (clove), 20.00 mg/mL (MIC), 40.00 mg/mL (MBC) for *Rosmarinus officinalis* L. (rosemary), and 20.00 mg/mL (MIC), > 40.00 mg/mL (MBC) for *Apium graveolens* subsp. *dulce* L. (celery) (Witkowska *et al*., 2013).

Table 1: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *P. cubeba* L. extract against *E. coli* O157:H7 (ATCC 43895).

	Ethanolic *P. cubeba* L. extract	Control (chlorhexidine)
MIC (mg/mL)	0.63 ± 0.00^{a}	0.08 ± 0.00^{a}
MBC (mg/mL)	1.25 ± 0.00^{A}	1.59 ± 0.05^{A}

Data are expressed as mean ± SD. Small letters represent significant differences of MIC between ethanolic *P. cubeba* L. extract and control groups at $p < 0.01$. Capital letters represent significant differences of MBC between ethanolic *P. cubeba* L. extract and control groups at $p < 0.01$.

E. coli killing time

Time-kill curve is frequently used to evaluate antimicrobial effect by monitoring the growth and death of bacteria over wide range of concentrations (Foerster *et al*., 2016). In the present work, the result for time-kill curve of the extract against *E. coli* O157:H7 (ATCC 43895) is plotted and presented in Figure 1. Based on the plotted curve, the numbers of bacteria for the sample incubated from 0 to 4 h without *P. cubeba* L. extract (0.0 × MIC) increased with time. The results were similar with the sample treated with 0.5 × MIC (0.31 mg/mL) with slight log reduction for the first half an hour of incubation. The findings shows the antibacterial activity of the extract; however, the concentration was not enough to prevent the growth of bacteria for the following 1 to 4 h.

In comparison with the samples treated with 1 × MIC (0.63 mg/mL), 2 × MIC (1.25 mg/mL) and 4 × MIC (2.50 mg/mL), the TPC decreased accordingly. The bacterial growths were inhibited at 0.63 mg/mL of the extract concentration. Bactericidal activity which referred to the MBC that kill 99.9% of the initial inoculum (Canillac and Mourey, 2001) and reduction of ≥ 3 $\log_{10}$ CFU/mL of the viable colony count (Scheetz *et al*., 2007) was seen in the sample treated with 2 × MIC incubated for 4 h with 3 log reduction. The finding shows that *P. cubeba* L. extract required 4 h to express the bactericidal activity against *E. coli* O157:H7 (ATCC 43895) at 1.25 mg/mL of concentration. In contrast, Ramli *et al*. (2017) had reported the bactericidal activity of *Syzygium polyanthum* (Wight) Walp. (Indian bay leaf) on *E. coli* at 4 × MIC (5.00 mg/mL) for 4 h of incubation time. Therefore, the results from the present work revealed that the bactericidal effect of *P. cubeba* L. extract was more effective to kill *E. coli* as it requires lower extract concentration. Another study by Zulfa *et al*. (2016) and Zainin *et al*. (2013) on *Cymbopogon citratus* (DC.) Stapf. (lemongrass) and *Boesenbergia rotunda* (L.) Mansf. (lesser galangal) have shown the bactericidal effect against *E. coli* at 2 × MIC (2.50 mg/mL) and 2 × MIC (0.04 mg/mL) at 2 h, respectively.

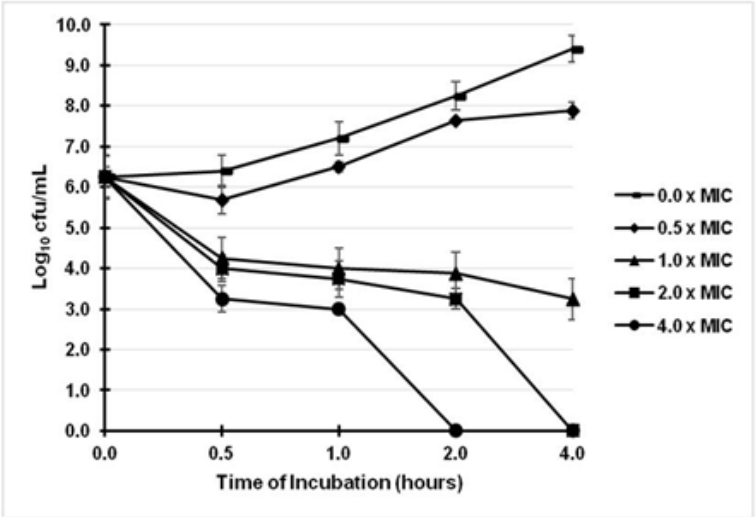

Figure 1: Time-kill curve plot of *E. coli* O157:H7 (ATCC 43895) for 0 × MIC, 0.5 × MIC, 1.0 × MIC, 2.0 × MIC and 4.0 × MIC of ethanolic *P. cubeba* L. extract at 0 to 4 h incubation time.

Bacterial load of untreated chicken meat samples

The enumeration of bacterial load of untreated raw chicken meat samples from wet market and supermarket were done by calculating the $\log_{10}$ CFU/g for TPC and *E. coli* count. Based on the findings, the bacterial load for wet market chicken was significantly higher as compared to the supermarket chicken. The TPC were recorded at 4.56 ± 0.26 $\log_{10}$ CFU/g for wet market and 3.60 ± 0.00 $\log_{10}$ CFU/g supermarket samples, and 3.60 ± 0.00 $\log_{10}$ CFU/g and 1.30 ± 0.00 $\log_{10}$ CFU/g for *E. coli* count of wet market and supermarket samples, respectively. In comparison with the study by Yusoff *et al*. (2015), the results for TPC and *E. coli* counts from chicken meat sample from wet market and supermarket showed no significant difference at 6.17 ± 0.02 and 5.90 ± 0.05, respectively for wet market, and 6.12 ± 0.01 and 5.97 ± 0.04, respectively for supermarket samples. However, the study shows higher bacterial contamination in the chicken meat samples from both types of retail outlets.

Generally, wet market is occupied by small-scale sellers, less equipped facilities, and concentrated with various kind of goods. Most of the sources of chicken meats are from free-range and small-scale farming which undergo backyard slaughtering without health inspection by veterinary authorities. The chicken meats are only substantially sterilised on healthy animal (Swartz, 2002). Without inspection, there is a possibility that the slaughtered chicken is a diseased chicken that may harvest pathogenic microorganisms. On the other hand, supermarkets are usually organised into aisles, clean, well ventilated, have well-equipped facilities and under-controlled environment. Systematic arrangement and hygienic conditions in supermarkets are better than those in the wet market (Adu-Gyamfi *et al*., 2012). All the chickens supplied to the supermarket were slaughtered at designated abattoir. In Malaysia, a number of designated and licensed abattoirs that complied with necessary food hygiene and sanitation requirement have been certified with Veterinary Health Mark (VHM) certification by the Department of Veterinary Services, Malaysia (DVS, 2012). This factor contributes to the bacterial load of the chicken meat samples. In the present work, even though the bacterial load obtained for wet market samples were significantly higher than supermarket samples; the bacterial level was still within the acceptable safety level. According to Fifteenth Schedule (Regulation 39, Table 1) of FOSIM (1985), the TPC level for unsafe chicken meat for consumption is above 10^6 CFU/g as compared to 10^4 CFU/g found in the present work.

Total plate count (TPC) of treated chicken meat samples

The TPC results for wet market and supermarket samples at 5 and 10 min exposure times stored at 4°C and -18°C for 0, 7 and 14 days are presented in Table 2. Statistically, the significant effect of *P. cubeba* L. extracts was detected in the treatment group and interaction between treatment groups with storage duration. Based on the findings, the TPC for the treated wet market and supermarket samples stored at 4°C showed identical bacterial survival trend. The bacterial load for the samples at 0.05%, 0.50% and 5.00% extract concentrations decreased upon application of the treatment at 5 and 10 min exposure times. However, the number of bacteria started to significantly increase from day 0 onwards. In preventing the spoilage of meat product, one of the most important factors is by controlling the storage temperature (Fung *et al*., 2008). Generally, storage of chicken meat product at 4°C will reduce the growth rate of the bacteria by minimising the chemical and biochemical reaction of the microorganisms. However, the recommended temperature is only acceptable for storage within one to two days (USDA, 2013). Specifically for chicken parts, FDA (2017) suggested the duration of storage is maximum for two days at refrigeration temperature (4°C), and nine months at freezer temperature (-18°C). In the present work, the samples treated with minimal extract concentration at 0.05% started to produce off-odour on day 7 for both wet market and supermarket samples at 7.26 ± 0.15 $\log_{10}$ CFU/g and 6.45 ± 0.21 $\log_{10}$ CFU/g, respectively. The bacterial counts for those samples exceeded the safe bacterial limits for fresh chicken meat at 10^6 CFU/g according to Fifteenth Schedule (Regulation 39, Table 1) of FOSIM (1985).

In contrast with the samples stored at -18°C, both treated wet market and supermarket chicken meat samples showed reduction of bacterial count upon storage. At 0.05% extract concentration, the TPC of the samples decreased until day 7, and started to increase for the following days. However, for the samples treated with 0.50% and 5.00% extract concentration, the bacteria count continuously decreased until day 14. For supermarket samples that has lower bacterial load than wet market samples, no macroscopic colonies were detected on the incubated PCA plate for the samples treated with 0.50% *P. cubeba* L. extract concentration in 10 min exposure time, and 5.00% extract concentration in 5 and 10 min exposure times, stored in freezer at day 14 of storage. Briefly, frozen storage is recognised as one of the cold preservation techniques that advantageously preserves the nutritional and sensory values of foods;

but the temperature does not eliminate harmful microorganisms, because as soon as temperature becomes favourable, the bacteria will resume their activity (Hiramatsu *et al*., 2005). However, according to Speck and Ray (1977), by lowering the temperature, it is able to control the microbiological activity during frozen storage by limiting the microbial growth and water activity (a_w). In addition Tedeschi and De Paoli (2011) also mentioned that at frozen temperature, most bacteria have poor survival rate due to the damage caused by ice crystal formation and electrolyte fluctuation. In the present work, with the addition of antibacterial activity of *P. cubeba* L., at 0.50% and 5.00% concentrations, the bacterial were unable to survive upon storage at frozen temperature for two weeks.

The antibacterial activity of *P. cubeba* L. also has been reported in various studies (Choi and Hwang, 2003; Silva *et al*., 2007; Singh *et al*., 2008). The research by Rukayadi *et al*. (2013) have shown the inhibition and bactericidal effect of *P. cubeba* L. against Pseudomonas aeruginosa, *E. coli*, and *Staphylococcus aureus* at 6.40, 3.20 and 1.60 mg/mL MIC and 12.80, 6.40 and 3.20 mg/mL MBC, respectively. The antibacterial activity of *P. cubeba* L. derivatives such as (-)-O-methylcubebin, (-)-O-benzylcubebin and (-)-hinokinin have also been reported effective against *Porphyromonas gingivalis* and *Bacteroides fragilis* with 50 µg/mL and 100 µg/mL MIC, respectively (Rezende *et al*., 2016). Another study by Khan and Siddiqui (2007) has also reported the effectiveness of antibacterial activity of *P. cubeba* L., *P retrofractum* Vahl., *P. longum* L. and *P. nigrum* L. against bacterial pathogens such as *Staphylococcus aureus*, *Salmonella* Typhi, *Pseudomonas aeruginosa*, *E. coli* and *Bacillus megaterium.*

E. coli count of treated chicken meat samples

E. coli is a rod-shaped, Gram-negative bacterium from the family of *Enterobacteriaceae*. Besides *Salmonella* spp., *E. coli* is another most important foodborne pathogens of public health interest

Table 2: Total plate count (TPC) of chicken meat samples treated with *Piper cubeba* L. stored at 4°C and -18°C for 0, 7 and 14 days storage time.

Storage temperature	Treatment group	Day 0		Day 7		Day 14	
		Exposure time (min)		Exposure time (min)		Exposure time (min)	
		5	10	5	10	5	10
4°C				*Wet market chicken*			
	Tap water	5.30 ± 0.00^{aA}	5.30 ± 0.00^{aA}	7.60 ± 0.00^{aA}	7.69 ± 0.13^{aA}	8.87 ± 0.39^{aA}	8.80 ± 0.28^{aA}
	0.00%	4.53 ± 0.16^{bA}	4.53 ± 0.04^{abA}	7.35 ± 0.09^{abA}	7.35 ± 0.05^{bA}	8.45 ± 0.21^{aA}	8.30 ± 0.00^{aA}
	0.05%	3.95 ± 0.07^{cA}	3.93 ± 0.46^{bcA}	7.26 ± 0.15^{bcA}	7.00 ± 0.10^{cA}	8.10 ± 0.13^{aA}	7.71 ± 0.01^{bB}
	0.50%	3.65 ± 0.07^{cdA}	3.62 ± 0.01^{cA}	6.70 ± 0.02^{cA}	6.87 ± 0.04^{cA}	8.04 ± 0.12^{aA}	7.52 ± 0.15^{bA}
	5.00%	3.48 ± 0.08^{dA}	3.46 ± 0.17^{cA}	5.73 ± 0.03^{dA}	5.65 ± 0.06^{dA}	7.71 ± 0.53^{aA}	6.49 ± 0.04^{cA}
				Supermarket chicken			
	Tap water	3.60 ± 0.00^{aB}	3.60 ± 0.00^{aA}	6.69 ± 0.55^{aA}	6.90 ± 0.43^{aA}	7.44 ± 0.13^{aA}	7.43 ± 0.02^{aA}
	0.00%	3.30 ± 0.00^{bA}	3.45 ± 0.21^{aA}	6.54 ± 0.34^{aA}	6.75 ± 0.21^{aA}	7.40 ± 0.14^{aA}	7.41 ± 0.05^{aA}
	0.05%	2.92 ± 0.12^{cA}	2.86 ± 0.05^{bA}	6.45 ± 0.21^{aA}	6.30 ± 0.00^{aA}	7.29 ± 0.30^{aA}	7.12 ± 0.10^{bA}
	0.50%	2.71 ± 0.04^{cdA}	2.68 ± 0.03^{bA}	6.25 ± 0.01^{aA}	6.19 ± 0.01^{abB}	7.00 ± 0.14^{aA}	6.96 ± 0.08^{bA}
	5.00%	2.66 ± 0.03^{dA}	2.56 ± 0.08^{bA}	5.69 ± 0.38^{aA}	5.39 ± 0.13^{bA}	6.84 ± 0.03^{aA}	6.18 ± 0.04^{cB}
-18°C				*Wet market chicken*			
	Tap water	5.30 ± 0.00^{aA}	5.30 ± 0.00^{aA}	5.45 ± 0.21^{aA}	5.54 ± 0.34^{aA}	5.62 ± 0.06^{aA}	5.61 ± 0.22^{aA}
	0.00%	4.53 ± 0.16^{bA}	4.53 ± 0.04^{abA}	4.70 ± 0.07^{bA}	4.75 ± 0.13^{bA}	4.89 ± 0.16^{bA}	4.93 ± 0.21^{aA}
	0.05%	3.95 ± 0.07^{cA}	3.93 ± 0.46^{bcA}	3.73 ± 0.03^{cA}	3.70 ± 0.00^{cA}	3.94 ± 0.06^{cA}	3.80 ± 0.17^{bA}
	0.50%	3.65 ± 0.07^{cdA}	3.62 ± 0.01^{cA}	3.63 ± 0.08^{cA}	3.60 ± 0.01^{cA}	3.58 ± 0.03^{dA}	3.48 ± 0.14^{bA}
	5.00%	3.48 ± 0.08^{dA}	3.46 ± 0.17^{cA}	3.45 ± 0.05^{cA}	3.38 ± 0.02^{cA}	3.40 ± 0.07^{dA}	3.26 ± 0.17^{bA}
				Supermarket chicken			
	Tap water	3.60 ± 0.00^{aB}	3.60 ± 0.00^{aA}	3.83 ± 0.02^{aA}	3.72 ± 0.08^{aA}	4.02 ± 0.02^{aA}	4.00 ± 0.03^{aA}
	0.00%	3.30 ± 0.00^{bA}	3.45 ± 0.21^{aA}	3.74 ± 0.09^{aA}	3.65 ± 0.07^{aA}	3.89 ± 0.09^{aA}	3.92 ± 0.02^{bA}
	0.05%	2.92 ± 0.12^{cA}	2.86 ± 0.05^{bA}	2.84 ± 0.34^{bA}	2.75 ± 0.21^{bA}	3.23 ± 0.04^{bA}	3.08 ± 0.00^{cB}
	0.50%	2.71 ± 0.04^{cdA}	2.68 ± 0.03^{bA}	2.45 ± 0.21^{bA}	2.30 ± 0.00^{cA}	2.30 ± 0.00^{cA}	0.00 ± 0.00^{dB}
	5.00%	2.66 ± 0.03^{dA}	2.56 ± 0.08^{bA}	2.30 ± 0.00^{bB}	2.30 ± 0.00^{cA}	0.00 ± 0.00^{dB}	0.00 ± 0.00^{dA}

The analysis was done using Tukey Post-Hoc Analysis. Data are expressed as mean ± SD. Small letters represent significant differences of TPC between exposure times at $p < 0.05$. Capital letters represent significant differences of TPC between treatment groups at $p < 0.05$.

implicated in chicken meat globally (Adeyanju and Ishola, 2014). It has been isolated worldwide from chicken meat and recommended to be totally absent from the meat before such can be considered fit for human consumption (Adesiji *et al*., 2011). As the bacterium is the intestinal microflora of the chicken, the presence of *E. coli* implies the cross-contamination of faecal material from the intestine to the meat during slaughtering (Singleton, 2004). The existence on the chicken meat may also be due to the unhygienic food preparation practice, environmental contaminants or from contaminated carcasses (USDA, 2015b). In the present work, the presence of *E. coli* was detected by the growth of green metallic sheen colonies on the incubated EMB agar. The results for *E. coli* count for wet market and supermarket samples at 5 and 10 min exposure times stored in 4°C and -18°C for 0, 7 and 14 days are presented in Table 3.

Similar with the TPC results, the statistical analysis for *E. coli* count showed significant effect of *P. cubeba* L. extract for treatment group and interaction between treatment groups with storage duration. There was no significant reduction of *E. coli* count between the samples treated with 5 and 10 min exposure times. For wet market samples stored at 4°C, the *E. coli* count significantly decreased after application of the extract at 0.05%, 0.50% and 5.00% concentrations. However, the *E. coli* count started to increase upon storage from day 0 to day 14. According to Nortjé *et al*. (1990), fresh meat is subjected to continuous refrigeration temperature during storage, but certain spoilage microorganisms are able to survive and grow at low temperature. Furthermore, as mentioned by McKellar *et al*. (2012), storage temperature is one of the important factors that affects the growth and distribution of *E. coli*. Most of *E. coli* survived after 22 to 24 months of storage at 4°C with original populations between

Table 3: *E. coli* count of chicken meat samples treated with *Piper cubeba* L. stored at 4°C and -18°C for 0, 7 and 14 days storage time.

Storage temperature	Treatment group	Day 0		Day 7		Day 14	
		Exposure time (min)		Exposure time (min)		Exposure time (min)	
		5	10	5	10	5	10
4°C				*Wet market chicken*			
	Tap water	3.60 ± 0.00^{aA}	3.69 ± 0.13^{aA}	3.78 ± 0.00^{aA}	3.81 ± 0.04^{aA}	4.06 ± 0.09^{aA}	4.19 ± 0.06^{aA}
	0.00%	3.60 ± 0.00^{aA}	3.66 ± 0.06^{aA}	3.70 ± 0.02^{aA}	3.69 ± 0.13^{aA}	4.01 ± 0.06^{aA}	4.08 ± 0.02^{aA}
	0.05%	2.94 ± 0.19^{bA}	2.92 ± 0.14^{bA}	3.26 ± 0.03^{bA}	3.20 ± 0.01^{bA}	3.66 ± 0.08^{bA}	3.54 ± 0.13^{bA}
	0.50%	2.86 ± 0.07^{bA}	2.81 ± 0.05^{bA}	3.19 ± 0.01^{bA}	3.14 ± 0.02^{bA}	3.52 ± 0.02^{bcA}	3.46 ± 0.11^{bA}
	5.00%	2.64 ± 0.00^{bA}	2.60 ± 0.03^{bA}	3.13 ± 0.11^{bA}	2.98 ± 0.10^{bA}	3.27 ± 0.10^{cA}	3.20 ± 0.08^{bA}
				Supermarket chicken			
	Tap water	1.30 ± 0.00^{aB}	1.30 ± 0.00^{aA}	1.30 ± 0.00^{aB}	1.30 ± 0.00^{aA}	1.69 ± 0.13^{aA}	1.84 ± 0.09^{aA}
	0.00%	1.30 ± 0.00^{bB}	1.30 ± 0.00^{bA}	1.30 ± 0.00^{bB}	1.30 ± 0.00^{bA}	1.45 ± 0.21^{aA}	1.60 ± 0.00^{bA}
	0.05%	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{cA}
	0.50%	0.00 ± 0.000^{dB}	0.00 ± 0.000^{dA}	0.00 ± 0.00^{dB}	0.00 ± 0.00^{dA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{cA}
	5.00%	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{cA}
-18°C				*Wet market chicken*			
	Tap water	3.60 ± 0.00^{aA}	3.69 ± 0.13^{aA}	3.60 ± 0.00^{aA}	3.69 ± 0.13^{aA}	3.88 ± 0.07^{aA}	3.99 ± 0.10^{aA}
	0.00%	3.60 ± 0.00^{aA}	3.66 ± 0.06^{aA}	3.45 ± 0.21^{aA}	3.45 ± 0.21^{aA}	3.70 ± 0.03^{aA}	3.73 ± 0.12^{aA}
	0.05%	2.94 ± 0.19^{bA}	2.92 ± 0.14^{bA}	2.37 ± 0.32^{bA}	2.20 ± 0.43^{bA}	2.49 ± 0.16^{bA}	2.38 ± 0.00^{bA}
	0.50%	2.86 ± 0.07^{bA}	2.81 ± 0.05^{bA}	2.21 ± 0.19^{bA}	2.13 ± 0.18^{bA}	2.11 ± 0.05^{cA}	1.30 ± 0.00^{cB}
	5.00%	2.64 ± 0.00^{bA}	2.60 ± 0.03^{bA}	1.30 ± 0.00^{cB}	1.30 ± 0.00^{bA}	1.30 ± 0.00^{dA}	0.00 ± 0.00^{dB}
				Supermarket chicken			
	Tap water	1.30 ± 0.00^{aB}	1.30 ± 0.00^{aA}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aA}
	0.00%	1.30 ± 0.00^{bB}	1.30 ± 0.00^{bA}	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}
	0.05%	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{dB}	0.00 ± 0.00^{dA}	0.00 ± 0.00^{dB}	0.00 ± 0.00^{dA}
	0.50%	0.00 ± 0.00^{dB}	0.00 ± 0.00^{dA}	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}
	5.00%	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bA}

The analysis was done using Tukey Post-Hoc Analysis. Data are expressed as mean ± SD. Small letters represent significant differences of *E. coli* count between exposure times at $p < 0.05$. Capital letters represent significant differences of *E. coli* count between treatment groups at $p < 0.05$.

10^3 and 10^4 CFU/disk (Hiramatsu *et al*., 2005). As in the present work, even though the extract expressed their antibacterial activity on the treated samples, the applied concentration with exposure time was still not enough to prevent the growth of *E. coli* upon storage at 4°C.

For frozen storage of wet market samples, the *E. coli* count continued to decrease after the treatment except for the samples treated with 0.05% extract concentration that started to increase from 2.37 ± 0.32 $\log_{10}$ CFU/g at day 7 to 2.49 ± 0.16 $\log_{10}$ CFU/g at day 14 for 5 min exposure time, and from 2.20 ± 0.43 $\log_{10}$ CFU/g to 2.38 ± 0.00 $\log_{10}$ CFU/g for 10 min exposure time. Frozen temperature is well established capable to avoid microbial growth. According to Casarin *et al*. (2009), the temperature is able to inactivate part of the bacterial population, and the magnitude of its effect will vary according to the type of food. In the present work, the antibacterial effect of *P. cubeba* L. extract was enhanced with the frozen storage temperature. However, 0.05% extracts concentration was still insufficient to keep reducing the *E. coli* count for wet market samples at frozen temperature until day 14.

For supermarket samples, all *E. coli* were unable to survive upon application of *P. cubeba* L. extract. At 4°C, only the control samples showed increase in bacterial number post day 7 of storage. While at -18°C, even the existing *E. coli* from the original load did not appear on the EMB agar at day 7 of storage most probably due to the growth limitation effect under frozen temperature. The antibacterial activity of *P. cubeba* L. on *E. coli* has been reported in several studies. Al-Tememy (2013) reported that aqueous extract produced higher antibacterial effect on *E. coli* and *P. aeruginosa* at 15 mm inhibition zone than *S. aureus* at 8 mm. For acetone and chloroform extracts, the inhibition zones were at 10 and 12 mm for *E. coli*, 13 and 10 mm for *P. aeruginosa*, and 16 and 11 mm for *S. aureus* respectively. Ethanolic extract of *P. cubeba* L. extract resulted in 10 mm (*E. coli*), 13 mm (*P. aeruginosa*) and 15 mm (*S. aureus*) inhibition zone. The metabolites of *P. cubeba* L. such as alkaloids, phenols and flavonoids contribute to the antibacterial activities of the extract. Upon treatment, the alkaloid will attach with bacterial DNA before exhibiting the bactericidal effect, while the phenol compound inhibits the microbial growth by precipitation activity on the microbial enzyme (Mar *et al*.,1991). Another antimicrobial mechanism as stated by Reed (1995) is through the composite of flavonoids hydroxyl group with cell wall proteins prior to break down the cell membrane of bacteria.

Sensory attributes acceptability evaluation

The evaluation of colour, odour, texture and overall acceptability were performed based on 9-point hedonic scale with the range of extremely liked (scale of 9) to extremely disliked (scale of 1), followed by statistical analysis. The result for means ± standard deviation for the scale is presented in Table 4. Based on the statistical analysis, the data shows no significant different ($p > 0.05$) between all the attributes tested. The results for colour, odour, texture and overall acceptability were at the range of 5 (neither like nor dislike) to 6 (slightly like) for all the samples treated with *P. cubeba* L. extract at different concentrations. Similar preferences were also obtained for the control samples treated with tap water and 0.00% extract concentration. However, the mean for samples treated with 0.05% extract concentration with 10 min exposure time showed the 'slightly like' score for all the attributes tested as compared to other samples. In the present work, it was

Table 4: Sensory attributes acceptability of raw chicken meat treated with *P. cubeba* L. at different exposure times.

Exposure time (min)	Treatment groups	Colour	Odour	Texture	Overall acceptability
5	Tap water	6.03 ± 1.69[a]	5.83 ± 1.60[a]	5.80 ± 1.54[a]	5.97 ± 1.56[a]
	0.00%	5.80 ± 1.54[a]	5.87 ± 1.78[a]	6.00 ± 1.51[a]	6.00 ± 1.55[a]
	0.05%	5.67 ± 2.02[a]	5.33 ± 1.67[a]	5.93 ± 1.68[a]	5.50 ± 1.43[a]
	0.50%	6.23 ± 1.46[a]	5.93 ± 1.64[a]	6.27 ± 1.44[a]	6.10 ± 1.40[a]
	5.00%	5.97 ± 1.65[a]	5.97 ± 1.67[a]	5.80 ± 1.71[a]	5.90 ± 1.54[a]
10	Tap water	5.57 ± 1.94[a]	5.60 ± 1.69[a]	5.60 ± 1.71[a]	5.53 ± 1.61[a]
	0.00%	6.07 ± 1.84[a]	5.73 ± 1.31[a]	5.97 ± 1.54[a]	6.00 ± 1.31[a]
	0.05%	6.07 ± 1.74[a]	6.33 ± 1.61[a]	6.33 ± 1.86[a]	6.30 ± 1.71[a]
	0.50%	5.73 ± 1.80[a]	5.70 ± 1.60[a]	5.60 ± 1.65[a]	5.77 ± 1.43[a]
	5.00%	5.93 ± 1.60[a]	5.67 ± 1.90[a]	6.33 ± 1.56[a]	5.97 ± 1.50[a]

The analysis was done using Tukey Post-Hoc Analysis. Data are expressed as mean ± SD. Small letters represent significant differences of sensory attributes acceptability of raw chicken meat treated with *P. cubeba* L. between treatment groups at $p < 0.05$.

shown that the application of *P. cubeba* L. extract on the chicken meat was neutral to positively accepted by the consumers in terms of all the attributes tested.

Conclusion

In conclusion, the antibacterial activity of the ethanolic extract of *P. cubeba* L. was effective against *E. coli*, and the application in chicken meat samples resulted in reduction of bacterial count. *P. cubeba* L. at the optimum condition can be used as one of the alternative approaches to reduce the bacterial load of raw chicken meat samples prior to cooking. As the chicken meat is categorised as perishable food and recognised as the main source of protein in Malaysian diet, the reduction in bacterial load is very important in ensuring the food safety as well as reducing the occurrence of foodborne poisoning associated with chicken meat.

References

Aamer, A. A., Abdul-Hafeez, M. M. and Sayed, S. M. 2014. Minimum inhibitory and bactericidal concentrations (MIC and MBC) of honey and bee propolis against multi-drug resistant (MDR) Staphylococcus sp. isolated from bovine clinical mastitis. Alternative and Integrative Medicine 3(4): 171.

Adesiji, Y. O., Alli, O. T., Adekanle, M. A. and Jolayemi, J. B. 2011. Prevalence of *Arcobacter*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. Sierra Leone Journal of Biomedical Research 3(1): 8-12.

Adeyanju, G. T. and Ishola, O. 2014. *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. SpringerPlus 3: 139.

Adu-Gyamfi, A., Torgby-Tetteh, W. and Appiah, V. 2012. Microbiological quality of chicken sold in Accra and determination of D10-value of E.coli. Food and Nutrition Sciences 3: 693-698.

Al Rdheha Ali, S. A., Al-daan, W. R. T. and Al Saffar, A. 2016. Antibacterial, anti-virulence factors of *Piper cubeba* extracts on *Escherichia coli* and *Staphylococcus saprophyticus* isolated from patient with urinary tract infection. International Journal of PharmTech Research 9(11): 216-228.

Al-Tememy, T. M. K. 2013. Antibacterial activity of *Piper cubeba* Linn. fruit extracts against selected bacterial pathogens in Basrah City. Basrah Journal of Veterinary Research 12(1): 142-151.

Andrews, J. M. 2001. Determination of minimum inhibitory concentration. Journal of Antimicrobial Chemotherapy 48(Suppl. S1): 5-16.

Brasil, I. M., Gomes, C., Puerta-Gomez, A., Castell-Perez, M. E. and Moreira, R. G. 2012. Polysaccharide-based multilayered antimicrobial edible coating enhances quality of fresh-cut papaya. LWT - Food Science and Technology 47(1): 39-45.

Canillac, N. and Mourey, A. 2001. Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria. Food Microbiology 18(3): 261-268.

Casarin, L. S., Tondo, E. C., Klein, M. P. and Brandelli, A. 2009. Survival of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* Enteritidis in frozen chicken hamburger. Journal of Muscle Foods 20(4): 478-488.

Centers for Disease Control and Prevention (CDC). 2017. *E. coli* (*Escherichia coli*). Retrieved on February 5, 2018 from CDC website: https://www.cdc.gov/ecoli/

Chang, W. S., Afsah-Hejri, L., Rukayadi, Y., Khatib, A., Lye, Y. L., Loo, Y. Y., ... and Son, R. 2013. Quantification of *Escherichia coli* O157:H7 in organic vegetables and chickens. International Food Research Journal 20(2): 1023-1029.

Choi, E. M. and Hwang, J. K. 2003. Investigations of anti-inflammatory and anti-nociceptive activities of *Piper cubeba*, *Physalis angulata* and *Rosa hybrid*. Journal of Ethnopharmacology 89(1): 171-175.

Clinical and Laboratory Standards Institute (CLSI). 2012. Reference method for performance standards for antimicrobial disk susceptibility tests; approved standard-eleventh edition, CLSI document M02-A11. USA: CLSI.

Clinical and Laboratory Standards Institute (CLSI). 2013. Reference method for dilution antimicrobial susceptibility tests, approved standard M7-A6. USA: CLSI.

Department of Veterinary Services (DVS). 2012. Manual Prosedur Veterinary Health Mark. Malaysia: Ministry of Agriculture and Agro-Based Industry.

Department of Veterinary Services (DVS). 2017. Livestock Statistics 2015/2016. Retrieved on September 23, 2017 from DVS website: http://www.dvs.gov.my/index.php/pages/view/1743

Efthymiopoulos, I., Hellier, P., Ladommatos, N., Kay, A. and Mills-Lamptey, B. 2019. Effect of solvent extraction parameters on the recovery of oil from spent coffee grounds for biofuel production. Waste and Biomass Valorization 10(2): 253-264.

Fatima, I., Waheed, S. and Zaidi, J. H. 2012. Essential and toxic elements in three Pakistan's medicinal fruits (*Punica granatum*, *Ziziphus jujuba* and *Piper cubeba*) analysed by INAA. International Journal of Food Sciences and Nutrition 63(3): 310-317.

Foerster, S., Unemo, M., Hathaway, L. J., Low, N. and Althaus, C. L. 2016. Time-kill curve analysis and pharmacodynamics modelling for *in vitro* evaluation of antimicrobials against *Neisseria gonorrhoeae.* BMC Microbiology 16: 216.

Food and Drug Administration (FDA). 2017. Refrigerator and freezer storage chart. Retrieved on February 10, 2018 from FDA website: https://www.fda.gov/downloads/Food/%20ResourcesForYou/HealthEducators%20/ucm109315.pdf

Food Safety Information System of Malaysia (FOSIM).

1985. P.U.(A) 437/85 Food Regulations 1985: Fifteenth Schedule (Regulation 39) Microbiological standard. Malaysia: FOSIM.

Fung, D. W. C., Edwards, J. and Crozier-Dodson, B. A. 2008. At-line methods for controlling spoilage. In Toldrà, F. (Ed). Meat Biotechnology, p. 289-318. USA: Springer.

Hiramatsu, R., Matsumoto, M., Sakae, K. and Miyazaki, Y. 2005. Ability of Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. Applied and Environmental Microbiology 71(11): 6657-6663.

Khan, M. and Siddiqui, M. 2007. Antimicrobial activity of Piper fruit. Natural Product Radiance 6(2): 111-113.

Kozačinski, L., Hadžiosmanović, M. and Zdolec, N. 2006. Microbiological quality of poultry meat on the Croatian market. Veterinarski Arhiv 76(4): 305-313.

Lawson, O. S., Oyewuni A., Ologunagba F. O. and Ojoma A. O. 2010. Evaluation of the parameters affecting the solvent extraction of soybean oil. APRN Journal of Engineering and Applied Sciences 5(10): 51-55.

Lim, T. K. 2012. *Piper cubeba*. In Lim, T. K. (Ed). Edible Medicinal and Non-Medicinal Plants Volume 4 - Fruits, p. 311-321. USA: Springer.

Mar, W., Tan, G. T., Gordell, G. A., Pezzutuo, J. M., Jurcic, K., Offermann, F., ... and Wagner, H. 1991. Biological activity of novel macrocyclic alkaloids from *Albizia amara* detected on the basis of interaction with DNA. Journal of Natural Products 54(6): 1531-1542.

McKellar, R. C., LeBlanc, D. I., Lu, J. and Delaquis, P. 2012. Simulation of *Escherichia coli* O157:H7 behavior in fresh-cut lettuce under dynamic temperature conditions during distribution from processing to retail. Foodborne Pathogens and Disease 9(3): 239-244.

Mead, G. C. 2004. Microbiological quality of poultry meat: a review. Brazilian Journal of Poultry Science 6(3): 135-142.

Mouid, M. G., Shakil Sait, S. and Kavimani, S. 2016. Effect of methanolic extract of *Piper cubeba* Linn. fruits on the pharmacodynamics of pioglitazone in alloxan induced diabetic rats. Indian Journal of Applied Research 6(2): 47-50.

Nahak, G. and Sahu, R. K. 2011. Phytochemical evaluation and antioxidant activity of *Piper cubeba* and *Piper nigrum.* Journal of Applied Pharmaceutical Science 1(8): 153-157.

Nortjé, G. L., Nel, L., Jordaan, E., Benderhorst, K., Goedhart, G., Holzapfel, W. H. and Grimbeek, R. J. 1990. A quantitative survey of a meat production chain to determine the microbial profile of a final product. Journal Food Protection 53(5): 411- 417.

Pandey, A. and Tripathi, S. 2014. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry 2(5): 115-119.

Parmar, V. S., Jain, S. C., Bisht, K. S., Jain, R., Taneja, P., Jha, A., ... and Boll, P. M. 1997. Phytochemistry of the genus Piper. Phytochemistry 46(4): 597-673.

Poste, L. M., MacKie, D. A., Butler, G. and Larmond, E. 1991. Laboratory methods for sensory analysis of food, publication 1864/E. Canada: Canada Publication Group - Publishing Centre.

Poultry World. 2014. Malaysian poultry industry meeting domestic demand. Retrieved on February 14, 2018 from Poultry World website: https://www.poultryworld.net/Meat/Articles/2014/3/Malaysian-poultry-industry-meeting-domestic-demand-1486503W/

Ramli, S., Radu, S., Shaari, K. and Rukayadi, Y. 2017. Antibacterial activity of ethanolic extract of *Syzygium polyanthum* L. (Salam) leaves against foodborne pathogens and application as food sanitizer. BioMed Research International (Article ID 9024246): 1-13.

Reed, J. D. 1995. Nutritional toxicology of tannins and related polyphenols in *Forage legnmes*. Journal of Animal Science 73(5): 1516-1528.

Rezende, K. C. S., Lucarini, R., Símaro, G. V., Pauletti, P. M., Januário, A. H., Esperandim, V. R., ... and Silva, M. L. A. E. 2016. Antibacterial activity of (−)-cubebin isolated from *Piper cubeba* and its semisynthetic derivatives against microorganisms that cause endodontic infections. Revista Brasileira de Farmacognosia 26(3): 296-303.

Rukayadi, Y., Lau, K. Y., Zainin, N. S., Zakaria, M. and Abas, F. 2013. Screening antimicrobial activity of tropical edible medicinal plant extracts against five standard microorganisms for natural food preservative. International Food Research Journal 20(5): 2905-2910.

Rukayadi, Y., Shim, J. S. and Hwang, J. K. 2008. Screening of Thai medicinal plants for anticandidal activity. Mycoses 51(4): 308-312.

Scheetz, M. H., Qi, C., Warren, J. R., Postelnick, M. J., Zembower, T., Obias, A. and Noskin, G. A. 2007. *In vitro* activities of various antimicrobials alone and in combination with tigecycline against carbapenem -intermediate or -resistant *Acinetobacter baumannii.* Antimicrobial Agents and Chemotherapy 51(5): 1621-1626.

Shafini, A. B., Son, R., Mahyudin, N. A., Rukayadi, Y. and Tuan Zainazor, T. C. 2017. Prevalence of *Salmonella* spp. in chicken and beef from retail outlets in Malaysia. International Food Research Journal 24(1): 437-449.

Silva, M. L., Coímbra, H. S., Pereira, A. C., Almeida, V. A., Lima, T. C., Costa, E. S., ... and Bastos, J. K. 2007. Evaluation of *Piper cubeba* extract, (-)-cubebin and its semi-synthetic derivatives against oral pathogens. Phytotherapy Research 21(5): 420-422.

Singh, G., Kiran, S., Marimuthu, P., de Lampasona, M. P., de Heluani, C. S. and Catalán, C. A. N. 2008. Chemistry, biocidal and antioxidant activities of essential oil and oleoresins from *Piper cubeba* (seed). International Journal of Essential Oil Therapeutics 2: 50-59.

Singleton, P. 2004. Bacteria in biology, biotechnology and medicine. 6th Ed. United Kingdom: John Wiley & Sons.

Speck, M. L. and Ray, B. 1977. Effects of freezing and storage on microorganisms in frozen foods: a review. Journal of Food Protection 40(5): 333-336.

Swartz, M. N. 2002. Human diseases caused by foodborne pathogens of animal origin. Clinical Infectious Diseases 34(Suppl 3): 111-122.

Talaro, K. P. and Chess, B. 2012. Foundations in Microbiology. 8th Ed. USA: McGraw-Hill Learning.

Tedeschi, R. and De Paoli, P. 2011. Collection and preservation of frozen microorganisms. Methods in Molecular Biology 675: 313-326.

United States Department of Agriculture (USDA). 2013. Basics for handling food safely. Retrieved on February 6, 2018 from USDA website: https://www.fsis.usda.gov/wps/wcm/connect/18cece94-747b-44ca-874f-32d69fff1f7d/Basics_for_Safe_Food_Handling.pdf?MOD=AJPERES

United States Department of Agriculture (USDA). 2015a. International egg and poultry review. Retrieved on February 14, 2018 from USDA website: https://www.ams.usda.gov/mnreports/ pywintlpoultryandegg.pdf

United States Department of Agriculture (USDA). 2015b. Chicken from farm to table. Retrieved on February 4, 2018 from USDA website: https://www.fsis.usda.gov/wps/wcm/connect/ad74bb8d-1dab-49c1-b05e-390a74ba7471/Chicken_from_Farm_to_Table.pdf

Witkowska, A., Hickey, D., Alonso-Gomez, M. and Wilkinson, M. 2013. Evaluation of antimicrobial activities of commercial herb and spice extracts against selected food-borne bacteria. Journal of Food Research 2(4): 37-54.

Yusoff, N. A. H., Noor, N. F. and Rukayadi, Y. 2015. Effects of Cosmos caudatus Kunth. (ulam raja) extract on microflora in raw chicken meat. International Journal of Current Microbiology and Applied Sciences 4(2): 426-435.

Zainin, N. S., Lau, K. Y., Zakaria, M., Son, R., Abdull Razis, A. F. and Rukayadi, Y. 2013. Antibacterial activity of *Boesenbergia rotunda* (L.) Mansf. A. extract against *Escherichia coli*. International Food Research Journal 20(6): 3319-3323.

Zulfa, Z., Chia, C. T. and Rukayadi, Y. 2016. *In vitro* antimicrobial activity of *Cymbopogon citratus* (lemongrass) extracts against selected foodborne pathogens. International Food Research Journal 23(3): 1262-1267.