Book Chapter

Antibacterial Activity of Ethanolic Extract of *Syzygium polyanthum* L. (*Salam*) Leaves against Foodborne Pathogens and Application as Food Sanitizer

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Abstract

The aim of this study was to determine antibacterial activity of *S. polyanthum* L. (*salam*) leaves extract foodborne pathogens. All the foodborne pathogens were inhibited after treated with extract in disk diffusion test with ranged $6.67 \pm 0.58 - 9.67 \pm 0.58$ mm of inhibition zone. The ranged of MIC values was between 0.63 to 1.25 mg/mL whereas MBC values was in ranged 0.63 mg/mL to 2.50 mg/mL. In time kill curve, *L. monocytogenes* and *P. aeruginosa* were found completely killed after exposed to extract in 1 h incubation at $4 \times$ MIC. Four hours had been taken to completely killed *E. coli*, *S. aureus*, *V. cholerae* and *V. parahaemolyticus* at $4 \times$ MIC. However, the population of *K. pneumoniae*, *P. mirabilis* and *S. typhimurium* only reduced to 3 Log CFU/mL. The treated cell showed cell ruptured and leakage of the cell cytoplasm in SEM observation. The significant reduction of natural microflora in grapes fruit were started at 0.50% of extract at 5 min and this concentration also parallel to sensory attributes acceptability where application of extract was accepted by the panellists until 5%. In conclusion, *S. polyanthum* extract exhibits antimicrobial activities, thus might be developed as natural sanitizer for washing raw food materials.

Keyword

Syzygium polyanthum L.; Antibacterial Activity; Foodborne Pathogens; Susceptibility Test; Food Sanitizer

Introduction

Food safety is a major concern for both consumers and food manufacturers alike. Even though the high degree of awareness of food preservation methods, the occurrence of disease outbreaks caused by foodborne pathogens and spoilage microorganisms in foods are still increasing [1]. Foodborne illness is also known as foodborne disease and colloquially referred to food poisoning is any illness resulting from the consumption of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food, rather than chemical or natural toxins. The symptoms for food poisoning are including diarrhea, fever, vomiting, abdominal pain and dehydration [2]. Currently to preserved food from spoilage, some manufacturers used synthetic antimicrobial agents to prevent the growth of food spoilage and food pathogenic microorganisms include benzoates, nitrates and nitrites [3]. However, emergences of microbial resistance to classic antimicrobial agents become a major health concern due to elevated use of chemical preservatives in food processing [4]. Nowadays, consumers more aware on food safety especially on the long term effect of synthetic additives in food including toxic or carcinogenic effect. Hence, this issue has led the increased of demand for high-quality, minimally processed foods with extended shelf-life and preferably free from or with a low level of synthetics additives in food [5]. Moreover, foods need to be safe, fresh with prolonged shelf-life. Therefore, antimicrobials agent from natural plants is a good source as an alternative to synthetic preservatives in order to satisfy consumers demand for safe and healthy food [6]. Antimicrobial agents can be either synthesized or occurred naturally in plant materials [7]. The main reasons for adding antimicrobial in food are to control food spoilage as well as to prevent the growth of foodborne pathogens [8]. This suggests that natural plants might be sources of antimicrobials agents that can be used to inhibit the growth of foodborne pathogens.

S. polyanthum L., which is synonym to *salam*, is a deciduous tropical tree belonging to the Myrtaceae family [9]. This plant grows wildy on lowlands and is widely distributed in the temperate, subtropical and tropical regions in the world [10].

This leaves had several name based on the location include, *S. polyanthum* in Malaysia and Indonesia is called *serai kayu* (Malay), *meselangan* is the name that used in Sumatra, *gowok* (Sunda), *salam* (Java, Sunda, Madura), *manting* (Java) or *kastolam* (Kangean) [11]. *S. polyanthum* leaves have been used traditionally as medicine or therapeutic agents including efficiency against ulcer, hypertension, diabetes, hyperuricemia, diarrheal, gastritis, skin diseases, and inflammation [11, 12]. Furthermore, *S. polyanthum* leaves were believed to possess antibacterial activity against *Streptococcus mutans* [11] and *Staphylococcus aureus* [13]. Besides that, this plant also had antifungal activities against spoilage fungi *Euroticum* spp., *Aspergillus* spp. and *Penicillium* spp. [14]. Furthermore, according to Perumal et al. [10], *S. polyanthum* leaves are also found to be non-cytotoxic to normal mammalian cell lines. Based on previous study, *S. polyanthum* leaves had antibacterial activity against *B. cereus* and *B. subtilis* [15].

Therefore the aim of this study was to determine the antimicrobial activity of *S. polyanthum* leaves extracts against a wide spectrum of foodborne pathogens.

Materials and Methods

Samples

Dried *S. polyanthum* leaves were purchased from Herbal Market Bandung, Indonesia, deposited and identify in Institute of Bioscience (IBS), Universiti Putra Malaysia.

Preparation of Extract

One hundred gram of dried *S. polyanthum* leaves were grounded using dry blender. Then, the samples were soaking in 400 mL absolute ethanol for seven days at room temperature as stated by Rukayadi et al. [16], with some modification. The mixture was then filtered using Whatman No. 2 filter paper and concentrated by using rotary evaporator at 50°C and speed of 150 rpm for 60 to 90 min. The extract was dissolved in 10% dimethylsulfoxide (DMSO) to obtain stock solution. The final concentration of

extract was standardized at 10 mg/mL or 1%. The stock solution was kept at -4°C.

Bacteria Cultures

A total of nine strains of frequently reported as foodborne pathogens were included *Escherichia coli* O157:H7 ATCC 43895, *Klebsiella pneumoniae* ATCC 13773, *Listeria monocytogenes* ATCC 19112, *Proteus mirabilis* ATCC 21100, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 29737, *Vibrio cholerae* (Isolate 2) and *Vibrio parahaemolyticus* ATCC 1780. All the microbial strains used in this study were maintained by sub-culturing them on the nutrient agar (NA) or nutrient agar mix with 3% of NaCl for *V. cholerae* and *V. parahaemolyticus* and incubated for overnight. Bacteria strains can be stored in this way for a few weeks on the agar plates before sub-culturing them again. While for the stock culture preparation, 0.5 mL of overnight culture with broth media were mixed into 0.5 mL of 50% sterile glycerol. Cultures were stored at -20°C. These stock cultures were kept until 6 months to 1 year [17].

Disk Diffusion Test

S. polyanthum extract was tested for antimicrobial activity using the disk diffusion method as described by CLSI [18]. Bacteria species with concentration in ranged 10^6 - 10^8 CFU/mL were spread on Mueller Hinton agar (MHA) with a sterile cotton swab. Sterile filter paper discs with 6 mm diameter were placed on top of the culture and 10 μ L of 10 mg/mL (w/v) of *S. polyanthum* leaves extract was loaded on the paper discs. 0.1% of commercial chlorhexidine (CHX) were used as positive control whereas 10% DMSO as negative control. The plates were incubated at 37°C for 24 hours. Evidence of clear zone indicates bacterial growth inhibition and the diameter were measured in mm.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Determination of MIC and MBC values were performed using a method described in the CLSI [18]. MIC was conducted in 96-well U-shaped microtiter plate using two fold standard broth microdilution method with an inoculum of approximately 10^6 - 10^8 CFU/mL. *S. polyanthum* leaves extract with concentration 10 mg/mL was mixed and two-folds diluted in the respective medium containing inoculum. Column 12 of the microtiter plate contained the highest concentration of extract (5 mg/mL) while column 3 contained the lowest concentration of extract (0.0097 mg/mL). Column 1 served as negative control (only medium, no inoculum, and no antimicrobial agent), meanwhile column 2 served as positive control for all samples (only medium and inoculum or antimicrobial agent-free well) for 24 hours. The MIC was defined as the lowest concentration of antimicrobial agent that able to inhibit the visible growth [16] while minimal bactericidal concentration (MBC) was stand for the lowest concentration of antimicrobial agent that completely kill the growth of culture. MBC was determined by sub-culturing the suspension (10 μ L) from each well in microtiter plate on MHA. The plates were then incubated at 37°C for 24 hours or until growth seen at positive control.

Time Kill Curve Assay

A time kill curve assay was carried out with the MIC values found previously in the micro plate bioassay, using a modification of the viable cells count method of de Souza et al. [19]. *S. polyanthum* leaves extract were diluted with the Muller Hinton broth (MHB) medium containing inoculum of approximately 10^6 - 10^8 CFU/mL to obtain final concentrations of 0 $\times$ MIC, 0.5 $\times$ MIC, 1 $\times$ MIC, 2 $\times$ MIC, and 4 $\times$ MIC for each bacterial species. At different time intervals of exposure, (0, 0.5, 1, 2, and 4 hours), 0.1 mL of the suspension was serially diluted in 1% phosphate buffered saline (PBS) and plated onto MHA. The plates were incubated at 37°C for 24 hours. The results were expressed in Log CFU/mL.

Scanning Electron Microscope (SEM)

Fresh *K. pneumoniae*, and *S. aureus* culture was treated with the extract and incubated at 37°C in MHB for 24 hours. The pellets were collected by centrifugation (5000 × g for 10 min) and were fixed with 2.5% glutaraldehyde for 4 - 6 hours at 4°C. Then, the pellets were washed with 0.1 M sodium cacodylate buffer for 10 min and were repeated for 3 times. The pellets were then post fixed with 1% osmium tetroxide for 2 hours at 4°C, washed again with 0.1 M sodium cacodylate buffer for 10 min and were repeated for 3 times. Then the pellets were dehydrated using 35, 50, 75 and 95% acetone for 15 min each. Lastly the pellets were dehydrated using 100% acetone for 15 min and were repeated for 3 times. Cell suspensions were transferred into a specimen basket, made from aluminium foil coated with albumin, and then put in critical dryer for 0.5 hours. The specimens were mounted on a stub and the sputter was coated with gold. The morphology of the cells were observed and images were obtained using SEM instrument.

Application of *S. polyanthum* Extract as Food Sanitizer on Grapes

The samples of grapes fruit (approximately 10 g) were treated with tap water and natural sanitizer with concentration of 0.05%, 0.50%, 1.00% and 5.00% of *S. polyanthum* extract according to Yusoff et al. [20] with slight modification. Grapes fruit were soaked separately at different time interval, 5, 10 and 15 min to determine their microflora growth viability. Untreated samples were remained unwashed. For bacteria growth determination, 1 mL from each treatment was diluted into 10^{-1} , 10^{-2} , and 10^{-3} dilution. Then, 0.1 mL from each dilutions series was spread on the different types of agar; Plate count agar, Eosin Methylene Blue agar (EMB) and Baird Parker agar and incubated at 37°C for 24 hours. The presence of colonies was counted.

Evaluation of Sensory Attributes Acceptability of Treated Grapes Fruit

The sensory evaluation acceptability test was performed as according to Brasil et al. [21], with slight modification. A group of 50 untrained panellists were presented with five different 3-digit coded samples placed in a random order. The evaluation was conducted based on the 9-point hedonic scale for inspection acceptance testing where panellists were assessed each treated samples in terms of colour (observe with eyes), odour (smell with nose) and the texture (touch with finger). The ratings for the each analysis of samples were given ranging scale from extremely disliked (scale of 1) to extremely liked (scale of 9).

Results

Yield of Extract

A 100 g of dried weight of *S. polyanthum* leaves were extracted using ethanol solvent and yielded 8.21 g of extract, which gave the percentage value of 8.21% total yield.

Disk Diffusion Test

The inhibition zone of *S. polyanthum* leaves extract against foodborne pathogens are shown in Table 1. The inhibition zones were between 6.67 ± 0.58 to 9.67 ± 0.58 mm. Results showed the inhibition zones of *S. polyanthum* extract were 7.00 ± 0.28 mm, 9.33 ± 0.50 mm, 9.67 ± 0.58 mm, 7.00 ± 0.32 mm, 6.67 ± 0.58 mm, 9.33 ± 0.58 mm, 6.67 ± 0.50 mm, 8.33 ± 0.58 mm, 6.67 ± 0.58 mm on *E. coli*, *K. pneumoniae*, *L. monocytogenes*, *P. aeruginosa*, *P. mirabilis*, *S. aureus*, *S. typhimurium*, *V. cholerae* and *V. parahaemolyticus* respectively. The larger inhibition zone gave the meaning of higher antibacterial activity of the extract on the tested microbial species.

Table 1: Inhibition zone of *S. polyanthum* L. leaves extract against foodborne pathogens.

Strains	Inhibition zone (mm)		
	<i>S. polyanthum</i> extract	CHX	DMSO
<i>E. coli</i> O157:H7	7.00 ± 0.28	9.00 ± 0.00	n.a
<i>K. pneumoniae</i>	9.33 ± 0.50	11.50 ± 0.50	n.a
<i>L. monocytogenes</i>	9.67 ± 0.58	12.00 ± 0.00	n.a
<i>P. aeruginosa</i>	7.00 ± 0.32	10.00 ± 0.51	n.a
<i>P. mirabilis</i>	6.67 ± 0.40	10.00 ± 0.70	n.a
<i>S. aureus</i>	9.33 ± 0.52	10.00 ± 0.23	n.a
<i>S. typhimurium</i>	6.67 ± 0.50	8.00 ± 0.00	n.a
<i>V. cholerae</i>	8.33 ± 0.30	8.80 ± 0.58	n.a
<i>V. parahaemolyticus</i>	6.67 ± 0.50	9.00 ± 0.00	n.a

n.a : No activity

Diameter of inhibition zones in mm (including disc)

Positive control (chlorhexidine: CHX; 0.1%); Negative control (DMSO; 10%)

Results were expressed as means ± standard deviation (SD); n = 3 × 3

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

From the result shown in Table 2, *S. polyanthum* leaves extract demonstrated broad-spectrum activity against all selected bacteria with the MIC values ranged 0.63 to 1.25 mg/mL. Among them *L. monocytogenes* and *S. aureus* were found to be the most susceptible pathogen with the MIC value of 0.63 mg/mL. Results show that the MBC were in ranged of 0.63 mg/mL to 2.50 mg/mL. *L. monocytogenes* gave the lower MBC values compared to other strains which was 0.63 mg/mL.

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. polyanthum* L. extract against foodborne pathogens.

Strains	MIC (mg/mL)	MBC (mg/mL)
<i>E. coli</i> O157:H7	1.25	2.50
<i>K. pneumoniae</i>	1.25	2.50
<i>L. monocytogenes</i>	0.63	0.63
<i>P. aeruginosa</i>	1.25	2.50
<i>P. mirabilis</i>	1.25	2.50
<i>S. aureus</i>	0.63	1.25
<i>S. typhimurium</i>	1.25	1.25
<i>V. cholerae</i>	1.25	1.25
<i>V. parahaemolyticus</i>	1.25	1.25

Time Kill Curve Assay

In this study, time-killing assay was done to find the correlation between the concentrations of *S. polyanthum* leaves extract with its killing effects on selected foodborne pathogens. Time-kill curve assay showed that *S. polyanthum* leaves extract can killed *L. monocytogenes* and *P. aeruginosa* at 4× MIC for 1 hours

[Figure 1(a) and (b)], while *E. coli*, *S. aureus*, *V. cholerae* and *V. parahaemolyticus* at 4× MIC for 4 hours [Figure 2(a), (b), (c) and (d)]. The population of *K. pneumoniae*, *P. mirabilis*, and *S. typhimurium* also shown a reduction < 3 Log₁₀ CFU/mL when treated with the extract at 4× MIC for 4 hours as shown at Figure 3(a), (b) and (c).

Figure 1(a): Time-kill curve plots for *L. monocytogenes* (0, 0.315, 0.630, 1.260, 2.520, mg/mL) following exposure to *S. polyanthum* L. extract. Values given in the brackets after species are 0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC, as respectively.

Figure 1(b): Time-kill curve plots for *P. aeruginosa* (0, 0.625, 1.250, 2.500, 5.000 mg/mL) following exposure to *S. polyanthum* L. extract. Values given in the brackets after species are 0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC, as respectively.

Scanning Electron Microscope (SEM)

Figure 4(a) and (b) show the treated and untreated *K. pneumoniae* cells with *S. polyanthum* extract at the concentration of 1.25 mg/mL for overnight. The untreated *K. pneumoniae* showed normal cells characteristics with rod shape and intact peptidoglycan layer. Meanwhile, after treated with *S. polyanthum* extract for overnight, cells appeared to be damaged with some irregularities surfaces, whereby the rod-shaped cells, shrank, deflated, and some of them were cavitated. Besides that, the effect of *S. polyanthum* extract against *S. aureus* is shown in Figure 5 (a) and (b). The grape-like cluster morphology of *S. aureus* was altered after the treatment. Disruptions with release of intracellular material associated to *S. aureus* cells losing their cytoplasm (empty and flaccid cells) were also observed.

Application of *S. polyanthum* Extract as Food Sanitizer on Grapes

Table 3 shows the effect of *S. polyanthum* against natural flora in grapes. Bacterial population which was detected in grapes includes *E. coli* and *S. aureus*. This study showed that, total plate count had been reduced significantly after exposed to 0.50% at 5 min soaking where the population decreased from 5.78 ± 0.05 to $5.19 \pm 0.13 \text{ Log}_{10} \text{ CFU/mL}$. On the other hand, *E. coli*'s population only had been significantly reduced after treated at 1.00% for 5 min and decreased to undetected at 5% extract at 5 min treatment while, *S. aureus* decreased to $\text{Log}_{10} 0.00 \pm 0.00 \text{ CFU/mL}$ started at 0.50% in 5 min.

Table 3: Effect of different concentrations and exposure times of *S. polyanthum* L. extract on natural microbial in grapes.

Sample	Grapes								
Bacterial species	TPC (Log ₁₀ CFU/mL)			<i>E. coli</i> (Log ₁₀ CFU/mL)			<i>S. aureus</i> (Log ₁₀ CFU/mL)		
ET / Treatment	5 min	10 min	15 min	5 min	10 min	15 min	5 min	10 min	15 min
Control	5.78± 0.05 ^{aA}	5.78 ± 0.05 ^{aA}	5.78±0.05 ^{aA}	4.14±0.05 ^{aA}	4.14±0.05 ^{aA}	4.14±0.05 ^{aA}	3.45±0.03 ^{aA}	3.45±0.03 ^{aA}	3.45±0.03 ^{aA}
Tap water	5.73± 0.08 ^{aA}	5.72± 0.03 ^{aA}	5.66±0.11 ^{aA}	4.11±0.05 ^{aA}	4.09±0.03 ^{aA}	4.05±0.04 ^{aA}	3.37±0.11 ^{aA}	3.14±0.07 ^{aA}	3.52±0.06 ^{aB}
0.05%	5.69± 0.04 ^{aA}	5.39± 0.03 ^{aA}	5.62±0.16 ^{aA}	4.08±0.06 ^{aA}	4.18±0.03 ^{aA}	4.10±0.02 ^{aA}	3.19±0.07 ^{bA}	3.33±0.18 ^{bA}	3.25±0.14 ^{bA}
0.50%	5.19± 0.13 ^{bA}	5.16± 0.14 ^{bA}	5.07±0.03 ^{bA}	3.88±0.18 ^{aA}	4.01±0.04 ^{aA}	3.95±0.06 ^{aA}	0.00±0.00 ^{cA}	0.00±0.00 ^{cA}	0.00±0.00 ^{cA}
1.00%	4.46± 0.22 ^{cA}	4.39± 0.12 ^{cAB}	3.03±0.07 ^{cB}	3.22±0.06 ^{bA}	3.46±0.19 ^{bAB}	3.11±0.07 ^{bB}	0.00±0.00 ^{cA}	0.00±0.00 ^{cA}	0.00±0.00 ^{cA}
5.00%	3.74± 0.03 ^{dA}	3.49± 0.23 ^{dA}	0.00±0.00 ^{dA}	0.00±0.00 ^{cA}	0.00±0.00 ^{cA}	0.00± 0.00 ^{cA}	0.00±0.00 ^{cA}	0.00±0.00 ^{cA}	0.00±0.00 ^{cA}

Values with different small letters within the same columns are significantly different ($p < 0.05$). Values with different capital letters within the same rows are significantly different ($p < 0.05$). ET: Exposure Time.

Table 4: Sensory attributes acceptability of treated grapes with *S. polyanthum* L. extract.

Attributes	Tap water	0.05%	0.50%	1.00%	5.00%
Colour	8.49 ± 0.88 ^a	8.16 ± 1.12 ^a	8.10 ± 1.08 ^a	7.65 ± 0.80 ^a	7.81 ± 1.88 ^a
Odour	8.59 ± 1.65 ^a	7.11 ± 1.11 ^a	7.53 ± 0.82 ^a	7.63 ± 1.90 ^a	7.25 ± 0.70 ^a
Texture	7.04 ± 0.89 ^a	7.10 ± 0.94 ^a	6.87 ± 1.02 ^a	7.68 ± 0.84 ^a	6.84 ± 2.10 ^a
Overall acceptability	7.24 ± 1.40 ^a	7.02 ± 1.51 ^a	7.13 ± 0.95 ^a	7.71 ± 1.79 ^a	7.28 ± 1.84 ^a

Mean values ± standard deviation with different small letters in the same row have significance different ($p < 0.05$).

Evaluation of Sensory Attributes Acceptability of Treated Grapes Fruit

Table 4 shows the sensory acceptability of treated grapes with *S. polyanthum* extract. Based on the result, it can be conclude that, most panellists were accepted this grapes samples which were washing with extracts and tap water with overall acceptability was more than scale 7. There also not significantly different between washing treatment using highest concentrations of extract (5%) and tap water. That means panellist does not able to differentiate between using tap water or extracts. Therefore, *S. polyanthum* did not affected the physical appearances of grapes.

Discussion

A recent trend in food processing is to avoid the use of chemical preservatives. Thus, natural antimicrobial alternatives are required. In this research, ethanol was used as a solvent. Ethanol also classified as a polar solvent. This means that this solvent is miscible in water and it will extract mostly the ionic compounds from *S. polyanthum* leaves. Ethanol has better dissolving capabilities compared to water because it has a slightly low dipole and dielectric; thus it is slightly polar [22]. Moreover, according to Marriott [23], the solvent permitted for use in the preparation of food ingredients are ethanol, ethyl acetate and acetone only.

From the disk diffusion result, *L. monocytogenes* gave the highest inhibition zone compared to others strain. On the other hand, *P. mirabilis*, *S. typhimurium* and *V. parahaemolyticus* observed to more resistant against the extract. Generally, in Gram-negative bacteria, their outer membranes serve as permeability barrier which allows only small hydrophilic molecules pass through into the cell, restricting their rate of penetration for certain antimicrobial compounds and excluding larger molecules. Besides, they also possess multidrug resistant pumps which exclude some of the antibacterial compounds across the barrier [24]. These special buildings make the Gram-negative bacteria more tolerant to any foreign compounds intake. On the other hand, disk diffusion test sometimes gave inaccurate

result due to some limitations such as the ability of extract to pass through the pore discs and the inability of hydrophobic compounds to diffuse into the media agar [25]. In addition according to Gangoue-Pieboji et al. [26], by using disc, some active compounds might be blocked in the disc pores and unable to pass through the inoculated media hence cannot express their activity. Besides that, inhibition zone of 0.1% of CHX against the pathogens was in ranged 8.80 ± 0.58 to 12.00 ± 0.00 mm. This finding showed lower inhibition zone compared study done by Abbas et al. [27], where mentions that the inhibition zone was between 13.84 ± 0.65 to 14.87 ± 0.53 mm on *E. faecalis* by using 2% of CHX. This observation maybe due to the difference concentration of CHX. However, according to Gupta et al. [28], inhibition zone of CHX against *P. aeruginosa* was 10.00 mm, whereas *S. aureus* was 11.00 mm. Therefore, the finding was similar with this present study. In conclusion, the disc-diffusion test is normally used as first screening in the detection of active compounds in plant extracts before further determination was performed.

L. monocytogenes and *S. aureus* were found to be the most susceptible pathogen with the MIC value of 0.63 mg/mL while the other strains shown 1.25 mg/mL. *L. monocytogenes* also showed the lower MBC values compared to other strains with 0.63 mg/mL. Besides that, *S. typhimurium*, *V. cholerae* and *V. parahaemolyticus* had same value for MIC and MBC, means this bacteria can be inhibited and killed at the same concentration of plant extract. This result showed that Gram-positive bacteria were easier to inhibit compared to Gram-negative. Gram-negative bacteria have a hydrophilic outer membrane which rich in lipopolysaccharide molecules. Therefore it serves as a penetration barrier towards macromolecules [29]. Although this description is widely accepted, and accepted for many essential oils, some researchers have stated that the Gram distinction may have little relation to growth inhibition and some herbs are equally effective against both groups of bacteria [30]. However, the outer membrane is not completely impermeable as there are porin proteins present in this layer that can create channels large enough to allow restricted passage of molecules with a molecular mass below 600 Da, such as substituted phenolics in herb

extracts and essential oils, allowing their slow penetration into the periplasmic space and the cytoplasmic membrane [31]. Thus it is possible that over a longer contact time the active compounds present in leaves extract would have the same effect on Gram-negative and Gram-positive bacteria [32]. Besides that, the *Euphorbia hirta* extract showed lower antimicrobial activity on *E. coli* compared to *S. polyanthum* extract with the MIC value of 3.13 mg/mL [33]. According to Rand et al. [34], *S. polyanthum* extract demonstrated better bactericidal and bacteriostatic properties compared to *B. oleracea* extract where MIC and MBC value of *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* were 100 mg/mL and 400mg/mL, 300 mg/mL and 400 mg/mL, 100 mg/mL and 200 mg/mL, 100 mg/mL and 400 mg/mL, respectively. *Moringa oliefera* seed extract display weaker antibacterial activity compared to *S. polyanthum* with MIC values was >4 mg/mL on *E. coli*, *P. aeruginosa* and *S. typhimurium* [35]. Moreover, *S. polyanthum* also shows good antibacterial effect than garlic and ginger extract. Based on Smith Palmer et al. [36], MIC and MBC of garlic and ginger extract on *L. monocytogenes*, *E. coli* and *S. aureus* were >1% whereas *S. polyanthum* gave bacteriostatic and bactericidal effect between 0.063% to 0.125% against the same bacteria strains. On the other hand, *S. polyanthum* and *Syzygium aromaticum* (clove) showed quite similar antibacterial activity. Inhibition zone of *S. aromaticum* against *E. coli*, *L. monocytogenes* and *S. aureus* were 9.7, 8.4 and 8.0 mm, respectively. Meanwhile the MIC values were 0.04%, 0.03% and 0.04% on the same pathogens. Besides that, *Syzygium cumini* showed no antimicrobial activity against *E. coli* and *K. pneumoniae*. However, it effectiveness against *S. aureus* with showed 9.00 mm in inhibition zone [37]. Therefore, *S. cumini* had lower antibacterial activity compared to *S. polyanthum* extract in term of disk diffusion test. According to Chikowe et al. [38], *Syzygium forte*, *Syzygium francisii*, *Syzygium moorei*, *Syzygium puberulum* and *S. wilsonii* illustrated weaker antibacterial activity compared to *S. polyanthum* where there was no inhibition zone against *P. mirabilis* and *S. aureus*. However, *S. francisii*, *S. moorei* and *S. wilsonii* showed higher inhibition zone against *E. coli* compared to *S. polyanthum* extract. On the other hand, all the tested *Syzygium* spp. gave lower antibacterial activity against *E. coli* compared to *S.*

polyanthum in term of MIC value except *S. francisii* with 0.256 mg/mL. Apart from that, *S. polyanthum* had higher antibacterial activity against *K. pneumoniae* than *S. forte*, *S. francisii*, *S. moorei*, *S. puberulum* and *S. wilsonii*.

Generally, different crude extracts show different antibacterial level among different microbes tested. These inconsistencies might be due to the different expression of the bioactive compounds present in the extracts. As suggested by Cowan [39], essential oils and polyphenolic compounds exhibited different bacteriostatic and bactericidal effect on bacterial strains. Therefore, minimum inhibitory concentration (MIC) is the parameter that commonly used to guide the selection on the antimicrobial agent used in treatment by predicting their efficacy at a standard inoculum approximately 10^6 CFU/mL after an incubation period of 18-24 hours [18]. However, MIC only provides limited information on the kinetics of the antimicrobial action. Due to this limitation, time-killing assay was performed in order to find the correlation between the rate of bactericidal activity with the incubation time and concentration of antimicrobial agent [32].

Figure 1(a) and (b) showed that both *L. monocytogenes* and *P. aeruginosa* had been completely killed at $4\times$ MIC in 1 hour incubation with 2.52 and 5.00 mg/mL of *S. polyanthum* leaves extract, respectively. These two strains were killed earlier compared to others. However finding by Penduka and Okoh [40], stated that *L. monocytogenes* can be killed completely with 0.314 mg/mL of crude *Garcinia kola* seed methanol extract in 0.5 hours of incubation. Therefore this finding suggested that *S. polyanthum* leaves extract might be possesses lower antibacterial activity against *L. monocytogenes*. In other case, according to Alwash et al. [41], *Melastoma malabathricum* extract had been reported able to kill completely *P. aeruginosa* at concentration 1.56 mg/mL within 8 hours. The comparison was hard to evaluate as both extracts were effective to kill *P. aeruginosa* completely at different concentration and incubation time. Generally, more concentrated extract will be able to kill bacteria in short period.

E. coli, *S. aureus*, *V. cholerae* and *V. parahaemolyticus* had been killed at 4× MIC within 4 hours as shown in Figure 2(a), (b), (c) and (d). Five mg/mL extract had been used to kill *E. coli* completely in 4 hours. In contrast, Mamman et al. [42], had reported the bactericidal activity of *Azadirachta indica* extract on *E. coli* strain was at concentration 250 mg/mL. Therefore, results from this study revealed that *S. polyanthum* leaves extract is a good antibacterial source against *E. coli* strain. According to Witkowska et al. [29], the bactericidal effect of sage extract on *S. aureus* was at concentration > 40 mg/mL for 24 hours of incubation time. In addition, rosemary and clove extracts able to kill *S. aureus* completely at 5 and 10 mg/mL concentration for 4 and 6 hours of incubation time, respectively. However, from this finding, *S. polyanthum* leaves extract only took 4 hours incubation to kill *S. aureus* completely at concentration 2.52 mg/mL. In comparison, *S. polyanthum* leaves have better bacterial effect against *S. aureus* compared to sage, rosemary and clove extract. Furthermore, *S. aureus* was Gram-positive bacteria where the membrane structure easier to disrupt compared to Gram-negative bacteria. From finding by Kwiecinski et al. [43], it stated that *S. aureus* can be killed within 15 min with 1% (v/v) of tea tree oil while El-Farmawi et al. [44], showed that methicillin-resistant *S. aureus* can be killed during 2 - 4 hours of incubation with cinnamon and green tea extract at concentration 300 µl/mL and 200 µl/mL, respectively. As conclusion, *S. polyanthum* leaves extract had a weaker bactericidal effect as compared to tea tree oil, cinnamon and green tea extracts. The time-kill plot obtained for *V. cholerae* and *V. parahaemolyticus* strains exhibited bactericidal end points which were at 4× MIC after 4 hours incubation. However, the population of both pathogens was reduced approximately to 3 Log at 2× MIC after 4 hours. Penduka et al. [40], reported that 69% of *V. parahaemolyticus* was killed at 5 mg/mL after 2 hours incubation using *Garcia kola* seed methanol extract. Therefore, *S. polyanthum* leaves have a quite similar bactericidal effect with *G. kola* seed extract where >50% *V. cholerae* and *V. parahaemolyticus*' population completely killed at same concentration and time incubation.

Figure 2(a): Time-kill curve plots for *E. coli* O157: H7 (0, 0.625, 1.250, 2.500, 5.000 mg/mL) following exposure to *S. polyanthum* L. extract. Values given in the brackets after species are 0x MIC, 0.5x MIC, 1x MIC, 2x MIC and 4x MIC, respectively.

Figure 2(b): Time-kill curve plots for *S. aureus* (0, 0.315, 0.630, 1.260, 2.520 mg/mL) following exposure to *S. polyanthum* L. extract. Values given in the brackets after species are 0x MIC, 0.5x MIC, 1x MIC, 2x MIC and 4x MIC, as respectively.

Figure 2(c): Time-kill curve plots for *V. cholerae* (0, 0.625, 1.250, 2.500, 5.000 mg/mL) following exposure to *S. polyanthum* L. extract. Values given in the brackets after species are 0x MIC, 0.5x MIC, 1x MIC, 2x MIC and 4x MIC, as respectively.

Figure 2(d): Time-kill curve plots for *V. parahaemolyticus* (0, 0.625, 1.250, 2.500, 5.000 mg/mL) following exposure to *S. polyanthum* L. extract. Values given in the brackets after species are 0x MIC, 0.5x MIC, 1x MIC, 2x MIC and 4x MIC, as respectively.

On the other hand, the population of *K. pneumoniae*, *P. mirabilis* and *S. typhimurium* were only reduced to <3 Log after 4 hours incubation as shown in Figure 3(a) and (b). According to

Supardy et al. [45], extract which able to reduce bacterial cell less than 3 log was indicated as having bacteriostatic effect. Therefore, the safety level for microbial population was below than 3 Log. According to El-Farmawi et al. [44], *K. pneumoniae* can be killed within 6 to 8 hours of incubation with cinnamon and green tea extract at concentration 500 µl/mL and 300 µl/mL, respectively. Its means *S. polyanthum* leaves had a quite weaker antibacterial activity compared to cinnamon. Research by Rajeh et al. [46], reported the bactericidal activity of *Euphorbia hirta* extract on *P. mirabilis* was at concentration 50 mg/mL at 24 hours incubation. Muniandy et al. [47] stated that concentration 1.08mg/mL of *Coleus aromaticus* extract can completely kill *P. mirabilis* within 24 hours of incubation time. On the other hand, Konate et al. [48], reported the bactericidal effect of *Sida alba* extract on *P. mirabilis* at concentration 0.05 mg/mL within 6 hours of incubation. Results revealed that *S. polyanthum* leaves possess better antibacterial agent compared to *E. hirta* and *C. aromaticus* extract, however this leaves extract showed lower bactericidal effect compared to *S. alba*. Foster [49] stated that *Salmonella* spp. had ability to adapt in wide range of conditions including able to grow in varies pHs and temperatures. Besides that, Mandal et al. [50], reported the reduction of *Salmonella* spp. until 2.19 log at concentration 0.512 mg/mL of *Camelia sinensis* extract within 24 hours of incubation time. Similarly, in this research, *Salmonella* spp. only reduced to 3 log and did not completely get killed after treatment with *S. polyanthum* leaves extract in 4-hour incubation. This showed that, *S. polyanthum* had better antibacterial activity against *Salmonella* spp. compared *C. sinensis* where the population reduction took only about 4 hours.

Figure 3(a): Time-kill curve plots for *K. pneumoniae* (0, 0.625, 1.250, 2.500, 5.000 mg/mL) following exposure to *S. polyanthum* L. extract. Values given in the brackets after species are 0x MIC, 0.5x MIC, 1x MIC, 2x MIC and 4x MIC, as respectively.

Figure 3(b): Time-kill curve plots for *P. mirabilis* (0, 0.625, 1.250, 2.500, 5.000 mg/mL) following exposure to *S. polyanthum* L. extract. Values given in the brackets after species are 0x MIC, 0.5x MIC, 1x MIC, 2x MIC and 4x MIC, as respectively.

Figure 3(c): Time-kill curve plots for *S. typhimurium* (0, 0.625, 1.250, 2.500, 5.000 mg/mL) following exposure to *S. polyanthum* L. extract. Values given in the brackets after species are 0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC, as respectively.

Increasing of plant extract's concentration will lead to diffusion into membrane cell thus caused membrane destruction [51]. In addition, the killing activity of *S. polyanthum* leaves extract was concentration-dependent. According to Mikusanti et al. [52], at higher concentration of extract, the membrane becomes leaky to cytoplasmic components which lead to cell death. It was also speculated that high concentrations of *S. polyanthum* leaves extract contribute to rapid killing of the microorganism because of the serious loss of membrane integrity and degenerative of cell wall. In order to kill the microorganisms, leaves extract need to bind, occupy and remain at the target site for sufficient period of time to prevent the metabolic process and interfere the chemical reactions of the bacteria. In addition, the increasing of plant extract can saturate the target site and cause rapid bactericidal effect [53]. The hydrophobicity of plants extract and their bioactive compounds contribute in the breaking down the membrane cells lipid and make them more permeable for the penetration [54]. Furthermore, the bioactive compounds in extract may inhibit the synthesis of essential metabolites (folic acid) by preventing the enzymatic reaction. The protein synthesis in the microorganisms also can be inhibited if the bioactive compounds interfere and changing the shape of ribosome. The

interference can lead to misreading of the genetic code on the mRNA [55].

Action modes of extract against tested strains were observed as shown in Figure 4 and Figure 5. The treated *K. pneumoniae* showed that the cell was ruptured and shrunk. This observation was supported by dos Santos et al. [56], where the electron-dense particles which stay packed in cytoplasm before, were dispersed and result an empty hollow in cytoplasm. This indicated that cytoplasm's compartment was release crossed the cell wall. Study conducted by Supardy et al. [45] also reported the damaged and distorted of *K. pneumoniae* cell after treated with 0.5 mg/mL of *Halimeda discoidea* extract for 12, 24 and 36 hours of treatment. Same result also obtained by Rajeshwari et al. [57] where the morphology of *K. pneumoniae* showed unusual shapes of expanding, swelling, shrinking, and other multiple disorientations that were absent in the control sets after treated with *H. discoidea* extract. In addition, same phenomenon was reported by Derakhshan et al. [58] who treated the *K. pneumoniae* with the cumin (*Cuminum cyminum* L.) herb extract. However, not all shrunked cells after treatment represent cells death. Some of them decrease their cell surface area as the way of adaptation, in order to minimize the target site for antimicrobial compounds to attach on them [45]. However, constant exposure of plant extract with increasing of concentration and extended time treatment will eventually kill the cells [29]. Generally, the results showed the shrinkage and deformation of the cells proved that the cells were under a suppressive and stressful environment. From the results, the prompt antibacterial action on the cells was seen to specifically attack the cell membrane components. Moreover, the treated cell of *S. aureus* also showed morphology changes. The grape-like cluster morphology of *S. aureus* was altered after the treatment. Disruptions with release of intracellular material associated to *S. aureus* cells losing their cytoplasm (empty and flaccid cells) were also observed. The distortion of the physical structure of the cell could cause the expansion and destabilization of the membrane and increase membrane fluidity, which in turn increases the passive permeability and manifest itself as a leakage of various vital intracellular constituents, such as ions,

ATP, nucleic acids, sugars, enzymes and amino acids. This observation may suggested that the ionic interactions between the cationic polymers and negatively charged lipopolysaccharides (as lipoteichoic acid, a component of the thick peptidoglycan layer of Gram-positive bacteria) in the outer membrane can be the responsible for the growth inhibition and lysis, through blockage of important nutrients flow such as Ca^{+2} and Mg^{+2} ion entering the cell [59].

Figure 4(a): Scanning electron micrograph of untreated *K. pneumoniae*.

Figure 4(b): Scanning electron micrograph of *K. pneumoniae* after treated with *S. polyanthum* L. extract at MIC value for 24 hours.

Figure 5(a): Scanning electron micrograph of untreated *S. aureus*.

Figure 5(b): Scanning electron micrograph of *S. aureus* after treated with *S. polyanthum* L. extract at MIC value for 24 hours.

Fresh food include fruit and vegetables may harbour a variety of microbes which priory originating from the environment where they grew. The microbes will keep growing along the postharvest handling and food processing and caused spoilage to the foods if no proper decontamination methods applied [60]. The growing and survival of these microbes with prolong time especially during storage period will spoil the foods and caused foodborne illness when consumed by people outside. As reported by Chang and Fang [61], the survivability of *E. coli* O157:H7 and *S. typhimurium* in shredded lettuce within 10 - 12 days imposed a potential health risk to consumers. In this study, treatment with tap water is referring to the common washing methods applied by household. There were some researchers who reported the capability of tap water to reduce the total bacterial count around 2 to 3 Log₁₀ CFU/mL [62] [63]. However, in this study, the treatment with tap water only showed slight reduction compared to previous study. Brackett [64] had reported that the use of tap water for washing cannot completely

remove the bacterial populations on food materials. Besides, there are limitations of using tap water in washing food materials which is due to the presence of chlorine residues in treated tap water. Chlorine residues have become a concern in food safety due to their potentiality to produce carcinogenic compounds such as trihalomethanes, haloacetic acids, halo ketones and chloropicrin when react with organic matter [65]. As stated by Gil and Badoni [66], re-using of processing water as sanitizer will make the tap water as another source of cross-contamination. In this study, the bacterial reduction in treated grapes fruit was proportional with the increasing of *S. polyanthum* extract concentration and soaking time. Research was in the similar of Abadias et al. [67] who also reported the reductions of microbial populations were increased as the concentration of sanitizer and washing time increased. However, study conducted by Tornuk et al. [68] proved that the ability of thyme sanitizer was affected by extract concentration while different exposure time did not gave significant reduction on the bacterial populations in apple fruits. Therefore, the relative influence in terms of microbial inactivation was: Tap water < 0.05% < 0.50% < 1.00 < 5.00%.

As stated by Vilgis [69], the ideal sanitizer is when the panellists are unable to recognize the difference between treated and non-treated samples which give the meaning of not much change occurred before or after the treatment applied. Study reported by Kumudavally et al. [70] reported the effectiveness of clove extract on reducing the pathogenic microflora in fresh mutton until 4 days treatment at $25 \pm 2^{\circ}\text{C}$, with at the same time gave no adverse effect on physical and sensory qualities. In correlation with that, Solomon et al. [71] had reported the organoleptic and chemical evaluations of suya (boneless meat pieces) after treated with basil extract for 30, 60, 90 and 120 mins. In their sensory analysis part, authors reported that the suya soaked with basil extracts enhance eating quality as it improved the flavour of meat. However, most of the panellists were not satisfied in terms of the final colour of treated suya (brownish green colour). In this study, grapes was accepted by panellist even though after treated with highest concentration of extract (5%). From this observation it can be concluded that, generally, the treated

samples which had been exposed to highest concentration and longest exposure time were accepted by panellist. That means, *S. polyanthum* extract did not affect or changes the physical characteristics of food samples after exposure to highest concentration of extract at maximum time of exposure.

Antimicrobial activity of herbs and spices varies widely, depending on the several factors including spices type, test medium, and types of pathogens. Moreover, microorganisms differ in their resistance to different types of spices and herbs. According to Kalemba and Kunicka [72], active components of herbs at low concentrations may interact synergistically with other factors including sodium chloride, acids and preservatives to increase preservation. However, antimicrobial activity of herb derived has been reported to diminish during food processing [73]. Therefore, further studies on the efficacy of these natural antimicrobial agents in a range of food products as well as evaluation of potential interactions of antimicrobial compounds with components of food matrices such as fats, carbohydrates and proteins are required.

Conclusion

In conclusion, susceptibility test is very important step in the screening of antibacterial activity of plant material. From the result, *S. polyanthum* leaves had antibacterial activity against wide spectrum of foodborne pathogens and able to reduce microflora count in fresh fruits. Therefore the plant might be promoted to further tests towards its evaluation as a sanitizer or preservative in wide range of foods.

References

1. J Meng, MP Doyle. Emerging and evolving microbial foodborne pathogens. Bulletin De Institut Pasteur. 1998; 96: 151-163.
2. GK Adak, SM Long, SJ O' Brien. Trends in indigenous foodborne disease and deaths, England and Wales. GUT. 2000; 51: 832-841.

3. GW Gould. Methods for preservation and extension of shelf life. *International Journal of Food Microbiology*. 1996; 33: 51-64.
4. CR Kiessling, JH Loftis, M Loftis, WM Kiessling, AR Datta, et al. Antimicrobial resistance of food-related *Salmonella* isolates. *Journal of Food Protection*. 2002; 65: 603-608.
5. DL Zink. The impact of consumer demands and trends on food processing. *Emerging Infectious Diseases*. 1997; 3: 467-469.
6. M Turgis, J Han, S Caillet, M Lacroix. Antimicrobial activity of mustard essential oil against *Escherichia coli* O157:H7 and *Salmonella typhi*. *Food Control*. 2009; 20: 1073-1079.
7. MA Gatto, I Ippolito, V Linsalata, NA Cascarano, F Nigro, et al. Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables. *Postharvest Biology and Technology*. 2011; 61: 72-82.
8. K Das, RKS Tiwari, DK Shrivastava. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*. 2004; 4: 104-111.
9. E Kato, R Nakagomi, MDPT Gunawan-Puteri, J Kawabata. Identification of hydroxychavicol and its dimers, the lipase inhibitors contained in the Indonesian spice, *Eugenia polyantha*. *Food Chemistry*. 2013; 136: 1239-1242.
10. S Perumal, R Mahmud, SP Piaru, LW Cai, S Ramanathan. Potential antiradical activity and cytotoxicity assessment of *Ziziphus mauritiana* and *Syzygium polyanthum*. *International Journal of Pharmacology*. 2012; 8: 535-541.
11. A Sumono, AS Wulan. The use of bay leaf (*Eugenia polyantha* Wight) in dentistry. *Dental Journal*. 2008; 41: 147-150.
12. A Ismail, M Mohamed, SA Sulaiman, WAN Wan Ahmad. Autonomic nervous system mediates the hypotensive effects of aqueous and residual methanolic extracts of *Syzygium polyanthum* (Wight) Walp. var. *polyanthum* leaves in anaesthetized rats. *Evidence-Based Complementary and Alternative Medicine: eCAM*. 2013; 2013, Article ID 716532, 16.
13. PW Grosvenor, A Supriono, DO Gray. Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2: antibacterial and

- antifungal activity. *Journal of Ethnopharmacology*. 1995; 45: 97-111.
14. ME Guynot, S Marin, L Sanchis, AJ Ramos. Screening for antifungal activity of some essential oils against common spoilage fungi of bakery products. *Food Science and Technology International*. 2005; 11: 25-32.
15. KY Lau, NS Zainin, F Abas, Y Rukayadi. Antibacterial and sporicidal activity of *Eugenia polyantha* Wight against *Bacillus cereus* and *Bacillus subtilis*. *International Journal of Current Microbiology and Applied Science*. 2013; 3: 499-510.
16. Y Rukayadi. JS Shim, JK Hwang. Screening of Thai medicinal plants for anticandidal activity. *Mycoses*. 2008; 51: 308-312.
17. N Varghese, PP Joy. Culture preservation techniques *Microbiology Laboratory Manual*. 2014; 31.
18. Clinical and Laboratory Standards Institute (CLSI). Reference method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. National Committee for Clinical Laboratory Standards. Wayne, Pennsylvania, USA. 2012.
19. EL Souza, TLM Stamford, EDO Lima. Sensitivity of spoiling and pathogen food-related bacteria to *Origanum vulgare* L. (Lamiaceae) essential oil. *Brazilian Journal of Microbiology*. 2006; 37: 527-532.
20. NAH Yusoff, NF Noor, Y Rukayadi. Effects of *Cosmos caudatus* Kunth. (Ulam raja) extract on microflora in raw chicken meat microflora in raw chicken meat. *International Journal Current Microbiology and Applied Science*. 2010; 4: 426-435.
21. IM Brasil, C Gomes, A Puerta-Gomez, ME Castell-Perez, RG Moreira. Polysaccharide-based multi-layered antimicrobial edible coating enhances quality of fresh-cut papaya. *LWT-Food Science and Technology*. 2012; 47: 39-45.
22. OA Fatoki, DA Onifade. Use of plant antimicrobials for food preservation. *International Journal of Veterinary and Agricultural Engineering*. 2013; 7: 1110-1113.

23. RJ Marriott. Greener chemistry preparation of traditional flavour extracts and molecules. *Agro Food Industry Hi-Tech*. 2010; 21: 46-48.
24. PA Lambert. Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria. *Journal of Applied Microbiology Symposium Supplement*. 2002; 92: 46-54.
25. H Othman, HS Wiart, C Khoo, TJ Lim, KN Ting. Optimal methods for evaluating antimicrobial activities from plant extracts. *Journal of Microbiological Methods*. 2011; 84: 161-166.
26. J Gangoue-Pieboji, N Eze, AJ Djintchui, B Ngameni, N Tsabang, et al. The in vitro antimicrobial activity of some traditionally used medicinal plants against beta-lactam-resistant bacteria. *The Journal of Infection in Developing Countries*. 2009; 3: 671-680.
27. AK Abbas, S Marjan, K Masoud, J Sharnaz. Stability of antibacterial activity of Chlorhexidine and Doxycycline in bovine root dentine. *Journal of Research in Pharmacy Practice*. 2014; 3: 19-22.
28. C Gupta, GP Amar, CU Ramesh. Antibacterial activity of amchur (dried pulp of unripe *Mangifera indica*) extracts on some indigenous oral microbiota causing dental caries. *Ethnobotanical Leaflets*. 2009; 13: 611- 617.
29. AM Witkowska, DK Hickey, M Alonso-Gomez, M Wilkinson. Antimicrobial activity of commercial herb and spices extracts against selected foodborne bacteria. *Journal of Food Research*. 2013; 2: 37-54.
30. P Kotzekidou, P Giannakidis, A Boulamatsis. Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate. *Food Science and Technology*. 2008; 41: 119-127.
31. T Abee, L Krockel, C Hill. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology*. 1995; 28: 169-185.
32. M Mueller, A Pena, H Derendorf. Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: kill curves

- versus MIC. *Antimicrobial Agents and Chemotherapy*. 2004; 48: 369-377.
33. EM Abu bakar. Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. *Journal of Medicinal Plants Research*. 2009; 3: 498-505.
 34. RH Rand, AS Abdulamir, SV Law, AB Fatimah, A Faridah, et al. Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. *The Open Microbiology Journal*. 2011; 5: 96-106.
 35. A Bukar, A Uba, TI Oyeyi. Antimicrobial profile of *Moringa oleifera* lam. extracts against some food – borne microorganisms. *Bayero Journal of Pure and Applied Sciences*. 2010; 3: 43-48.
 36. A Smith-Palmer, J Stewart, L Fyfe. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Applied Microbiology*. 1998; 26: 118-122.
 37. GF de Oliveira, NAJC Furtado, AAS Filho, CHG Martins, JK Bastos, et al. Antimicrobial activity of *Syzygium cumini* (Myrtaceae) leaves extract. *Brazilian Journal of Microbiology*. 2007; 38: 381-384.
 38. G Chikowe, L Mpala, IE Cock. Antibacterial activity of selected Australian *Syzygium* species. *Pharmacognosy Communications*. 2013; 3: 1-7.
 39. MM Cowan. Plant products as antimicrobial agents. *Clinical microbiology reviews*. 1999; 12: 564-582.
 40. D Penduka, AI Okoh. In vitro antilisterial properties of crude methanol extracts of *Garcinia kola* (heckel) seeds. *The Scientific World Journal*. 2012; 12: 6952-6965.
 41. MS Alwash, N Ibrahim, WY Ahmad. Identification and mode of action of antibacterial components from *Melastoma malabathricum* Linn. Leaves. *American Journal of Infectious Diseases*. 2013; 9: 46-58.
 42. PH Mamman, WP Mshelia, SC Susbatrus, S Sambo. Antibacterial effects of crude extract of *Azadirachta indica* against *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus*. *International Journal of Medicine and Medical Sciences*. 2013; 5: 14-18.

43. J Kwiecinski, S Eick, K Wojcika. Effects of tea tree (*Melaleuca alternifolia*) oil on *Staphylococcus aureus* in biofilms and stationary growth phase. *International Journal of Antimicrobial Agents*. 2009; 33: 343-347.
44. D El-Farmawi, Z Olama, H Holail. The antibacterial effect of some natural bioactive materials against *Klebsiella pneumoniae* and MRSA. *International Journal of Current Microbiology Applied Science*. 2014; 3: 576-588.
45. S Supardy, ND Afifah, SF Ibrahim, NZ Zakaria. Inhibition of *Klebsiella pneumoniae* ATCC 13883 by hexane extract of *Halimeda discoidea* (Decaisne) and the identification of its potential bioactive compounds. *Journal of Microbiology and Biotechnology*. 2012; 22: 872-881.
46. MAB Rajeh, Z Zuraini, S Sasidharan, LY Latha, S Amutha. Assessment of *Euphorbia hirta* L. leaf, flower, stem and root extracts for their antibacterial and antifungal activity and brine shrimp lethality. *Molecules*. 2010; 15: 6008-6018.
47. K Muniandy, Z Hassan, MHM Isa. Antimicrobial activity of ethanolic extract of *Coleus aromaticus* against common wound pathogens, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2013; 5: 1286.
48. K Konate, A Mavoungou, JF Lepengue. Antimicrobial activity of polyphenol-rich fractions from *Sida alba* L. (Malvaceae) against co-trimoxazol-resistant bacteria strains. *Annals of Clinical Microbiology and Antimicrobials*. 2012; 11: 2-6.
49. JW Foster. Low pH adaptation and the acid tolerance response of *Salmonella typhimurium*. *Critical Reviews in Microbiology*. 1995; 21: 215-237.
50. S Mandal, M DebMandal, NK Pal, KJ Saha. Inhibitory and killing activities of black tea (*Camellia sinensis*) extract against *Salmonella enterica* serovar Typhi and *Vibrio cholerae* O1 biotype El Tor serotype Ogawa isolates. *Jundishapur Journal of Microbiology*. 2001; 4: 115-121.
51. AS Thangiah, J Obey, NC Mutuku. Phytochemical analysis of *Vernonia adoensis* leaves and roots used as a traditional medicinal plant in Kenya. *International Journal of Pharmaceutical and Biological*. 2013; 3: 46-52.
52. M Mikusanti, BSL Jenie, R Syarief, B Pontjo, BGT Mulyadi. Antibacterial activity of temu kunci tuber (*Kaempheria*

- pandurata) essential oil against *B. cereus*. Medical Journal of Indonesia. 2009; 18: 10-17.
53. G Satta, G Cornaglia, A Mazzariol, G Golini, S Valisena, et al. Target for bacteriostatic and bactericidal activities of β -lactam antibiotics against *Escherichia coli* resides in different penicillin-binding proteins. Antimicrobial Agents Chemotherapy. 1995; 39: 812-818.
 54. J Sikkema, JAM Bont, B Poolman. Mechanisms of membrane toxicity of hydrocarbons. Microbiology Review. 1995; 59: 201-222.
 55. GJ Tortora, BR Funke, CL Case. Antimicrobial drug. In Microbiology: an introduction. San Francisco: Pearson Benjamin Cumming. 2007.
 56. ECG dos Santos, CL Donnici, ERS Camargos. Effects of *Copaifera duckei* Dwyer oleoresin on the cell wall and cell division of *Bacillus cereus*. Journal of Medical Microbiology. 2013; 62: 1032-1037.
 57. H Rajeshwari, S Nagveni, A Oli, D Parashar, KR Chandrakanth. Morphological changes of *Klebsiella pneumoniae* in response to cefotaxime: A scanning electron microscope study. World Journal of Microbiology and Biotechnology. 2009; 25: 2263- 2266.
 58. S Derakhshan, M Sattari, M Bigdeli. Effect of subinhibitory concentrations of cumin (*Cuminum cyminum* L.) seed essential oil and alcoholic extract on the morphology, capsule expression and urease activity of *Klebsiella pneumoniae*. International Journal of Antimicrobial Agents. 2008; 32: 432-436.
 59. J Diaz-Visurraga, G Cardenas, A Garcia. Morphological changes induced in bacteria as evaluated by electron. Microscopy: Science, Technology, Applications and Education. 2010; 307-315.
 60. MR Wachtel, AO Charkowski. Cross-contamination of lettuce with *Escherichia coli* O157: H7. Journal of Food Protection. 2002; 65: 465-470.
 61. JM Chang, TJ Fang. Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7. Food Microbiology. 2007; 24: 745-751.

62. DO Ukuku, WE Fett. Method of applying sanitizers and sample preparation affects recovery of native microflora and Salmonella on whole cantaloupe surfaces. *Journal of Food Protection*. 2004; 67: 999-1004.
63. S Inatsu, O Ayumi, N Kumiko. Idebenone acts against growth of *Helicobacter pylori* by inhibiting its respiration. *Antimicrobial Agents and Chemotherapy Journal*. 2006; 50: 2237-2239.
64. RE Brackett. Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. *Journal of Food Protection*. 1992; 55: 808-814.
65. RG Klaiber, S Baur, G Wolf, WP Hammes, R Carle. Quality of minimally processed carrots as affected by warm water washing and chlorination. *Innovative Food Science and Emerging Technologies*. 2005; 6: 351-362.
66. CO Gill, M Badoni. Recovery of bacteria from poultry carcasses by rinsing, swabbing or excision of skin. *Food Microbiology*. 2005; 22: 101-107.
67. M Abadias, TP Canamas, A Asensio, M Anguera, I Vinas. Microbial quality of commercial 'Golden Delicious' apples throughout production and shelf-life of Lleda (Catalonia, Spain). *International Journal of Food Microbiology*. 2006; 108: 404-409.
68. F Tornuk, H Cankurt, I Ozturk, O Sagdic, O Bayram, et al. Efficacy of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on fresh cut carrots and apples. *International Journal of Food Microbiology*. 2011; 148: 30-35.
69. TA Vilgis. Texture, taste and aroma: multi-scale materials and the gastrophysics of food. *Flavour*. 2013; 2: 2-5.
70. KV Kumudavally, A Tabassum, K Radhakrishna, AS Bawa. Effect of ethanolic extract of clove on the keeping quality of fresh mutton during storage at ambient temperature ($25 \pm 2^\circ\text{C}$). *Journal of Food Science and Technology*. 2011; 48: 466-471.
71. EB Solomon, S Yaron, KR Matthews. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Applied of Environmental Microbiology*. 2002; 68: 397-400.

72. D Kalembe, A Kunicka. Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*. 2003; 10: 813-829.
73. NS Weerakkody, N Caffin, N Turner, GA Dykes. In vitro antimicrobial activity of less-utilized spice and herb extracts against selected food-borne bacteria. *Food Control*. 2010; 21: 1408-1414.