International Food Research Journal 21(1): 379-386 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

In vitro antimicrobial activity of musk lime, key lime and lemon extracts against food related pathogenic and spoilage bacteria

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Article history

Received: 17 September 2012
Received in revised form: 5 September 2013
Accepted: 6 September 2013

Keywords

Aeromonas veroni
Ethanol extract
Key lime
Musk lime
Pseudomonas fluorescens

Abstract

The demand for novel antimicrobial agents from natural resources has been increased worldwide for food conservation purpose. In this study antimicrobial activity of musk lime, key lime and lemon were evaluated against various food borne pathogens and spoilage bacteria using disc diffusion test. Type of extraction solvent and concentration level significantly influenced the antibacterial activity of all the extracts. Ethanol extracts of musk lime, key lime and lemon exhibited significant broadest inhibitory activity at 100% concentration level (pure extract) compared to water and juice extracts. 100% ethanol extracts of musk lime (39.7 mm), key lime (26.7 mm) and lemon (32.0 mm) exhibited the largest diameter of inhibition zone (DIZ) against *Aeromonas veronii.* 100% water extracts of musk lime (25.3 mm), key lime juice extract (23.3 mm) and water extracts of lemon (23.7 mm) was most effective against food spoilage bacteria, *A. veronii*. The prominent results of the antimicrobial activity from lime, key lime and lemon extracts may attribute them as potential natural food preservatives and could be used in pharmaceuticals field.

Introduction

Occurrence of bioactive compounds in plants or spices/herb extracts is the basis to the antimicrobial effects which at time protects the plant themselves against microorganisms, fungus and viral infectivity (Tagoe *et al*., 2010). Nowadays natural antimicrobials received a compact awareness from a series of issues related to microorganisms control and as a source of pharmaceutical active compounds (Amrita *et al.*, 2009; Tagoe *et al*., 2010; Tajkarimi *et al.*, 2010). Alternatively as the safety aspects of chemical or synthetic food additives being questioned, the demand for naturally occurring preservatives has been increased worldwide (Chanthaphon *et al.*, 2008; Pundir and Jain, 2010). The exploration of novel antimicrobial agents from natural resources inclusive of plant or plant based products (spice or herbs) and others has been used mainly for curing illness, food safety and food conservation purpose since many years ago (Hammer *et al.*, 1999; Belletti *et al.*, 2004; Fajimi and Taiwo, 2005; Tagoe *et al*., 2010).

Earlier ancient civilization believed that certain herbs have remedial supremacy (Onyeagba *et al*., 2005; Nkambule, 2008). Nations like India one of the developing states fulfils the requirement of medicinal sources mainly from plants to cure infectious diseases (Amrita *et al*., 2009). Egyptians implicated plant extracts for preserving the dead (Nkambule, 2008). Africans practiced plant medicines for the treatment of many diseases and infections (Aboaba *et al*., 2006). The oldest and greatest continual history has been recorded in China as the country implicated herbs practices for more than 5000 years ago. Concurrently, many herbs/spices and plant derived products have been used extensively in medicinal field from ancient era to present. It has been proven to cure certain illness in replacement of chemical compounds or antibiotics particularly in many Asian, African and other countries. By referring to World Health Organization (WHO) about 80% of world requirements on medicines for health needs attained from botanical preparations (plant extracts or their bioactive compounds) (Indu *et al*., 2006; Mohanta *et al*., 2007; Nkambule, 2008; Hema *et al*., 2009; Tagoe *et al*., 2010; Yan and Asmah, 2010).

Compilation of data proves that natural antimicrobials/antioxidants, fruits and vegetables extracts have significant consideration to be used in food as food additives or natural preservative with intention for multidimensional quality (flavour, aroma) and nutritional level enhancement as well antimicrobial property (Belletti *et al.*, 2004; Nanasombat and Lohasupthawee, 2005; Hoque *et al.*, 2007; Corbo *et al*., 2008; Nkambule, 2008; Amrita *et al*., 2009; Weerakkody *et al.*, 2010; Das *et al.*, 2011). Exploration on natural preservatives rose even with exclusive knowledge on how these preservative agents' works on mechanism, toxicological and sensory consequences essentially in food substances

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(Tajkarimi *et al*., 2010; Nirmal and Benjakul, 2011). Additionally, the usage of spices and herbs has been increased in all cultures of developed countries as well due to its beneficial effects (Xing *et al*., 2010). The main advantages of natural antimicrobial agents were focused in the field of food safety, quality and preservation; as a controlling agent for microbial contamination in food (food safety), lengthen food products shelf life by removal of unpleasant food borne pathogens/ spoilage bacteria (food preservation), retard decolourization and retain colour, texture, flavour, and nutritional value (food quality), reduce the usage of synthetic chemical preservatives (food safety) (Belletti *et al*., 2004; Amrita *et al.*, 2009; Garg *et al*., 2010; Tajkarimi *et al*., 2010; Weerakkody *et al.*, 2010).

Various citrus fruits have been reported for its antimicrobial effect inclusive of both gram negative and gram positive bacteria (Owhe-Ureghe *et al*., 2010). Moreover, citrus fruits intake has been linked towards lesser risk of several chronic diseases (Nagy, 1980). Lime, lemons, mandarins and oranges are some of the important fruits included in the genus of Citrus (Chanthaphon *et al*., 2008). Lime, key lime and lemons were accepted as food ingredient mainly for flavouring intention as well to add on the acidity (Tomotake *et al*., 2006). Lime consist of the nutritional profile of carbohydrates, vitamins, minerals, soluble and insoluble fiber, sugar, sodium, fatty acids, amino acids and others (Jayana *et al.*, 2010); It is well known as one of the fundamental constituent in the mixture of most herbals. Lemon (Citrus limon) reveals nutritional profile of citric acid, ascorbic acid, flavonoids, minerals and others. Their roles as food ingredient were mainly in the production of jams, soft drinks and alcoholic drinks (Miyake *et al*., 1997). Lime and lemon juice extracts have exhibited wide range of DIZ against all strains of *Pseudomonas aeruginosa* tested by (Adedeji *et al.*, 2007). The DIZ were in the range of 7mm to 21.5 mm towards these gram negative bacteria which possibly owing to its active compounds (Adedeji *et al.*, 2007). Likewise, lime crude extract reported to be very effectual against *Vibrio cholerae* (28 mm) compared to the other gram negative bacteria tested, *Enterobacter* (9 mm), *Citrobacter* (8 mm), *E. coli* (8 mm) (Jayana *et al*., 2010). To our knowledge, there are no reports for the effect of different solvents in the extraction method for antibacterial activity of musk lime, key lime and lemon on food borne pathogens and spoilage bacteria. Thus, the aim of this research was to assess the *in vitro* antimicrobial activity of ethanol, water and juice extracts of musk lime, key lime and lemon against various food related pathogenic and spoilage bacteria.

Materials and Methods

Test microorganisms

Microbial strains used in this study includes *Salmonella typhimurium*, *Salmonella typhi*, *Staphylococcus aureus*, *E. coli*, *Listeria monocytogenes*, *Aeromonas hydrophila*-like DNA group 2, *Aeromonas hydrophila*, *Aeromonas media*-like DNA group 5B, *Aeromonas sobria* DNA group 7, *Aeromonas sobria* DNA group 8, *Aeromonas veronii*, *Aeromonas veronii* DNA group 10, *Pseudomonas fluorescens*, *Pseudomonas lundensis*, *Carnobacterium maltaromaticum*, *Yersinia ruckeri* and *Psychrotrophic immobilis*. All the cultures were obtained from Microbiological Laboratory of Faculty of Food Science and Technology, Universiti Putra Malaysia. Bacterial cultures were grown and maintained on Tryptic Soy Agar (TSA). They were sub cultured on every two weeks interval and subsequently stored at 4°C.

Plant materials and preparation of the extract

Musk lime, key lime and lemon were obtained from local market in Kuchai Lama, Selangor. These plant parts were selected on the basis of their use as food preservatives (Pundir and Jain, 2010). The fresh samples were thoroughly washed with sterile distilled water and surface sterilised with ethanol. Ethanol on the sample surface was allowed to evaporate under laminar flow. The samples were further subjected for various extractions (juice, ethanol and water) procedures.

Juice extraction was prepared according to methods reported by Owhe-Ureghe *et al.* (2010). Samples were cut into halves using sterile knife and the liquid was squeezed individually in sterile container. This extract was marked as 100% concentrated juice extract. Concentration of 50% was prepared by diluting the 100% extract with right volume of sterilized distilled water.

Ethanol and water extraction were prepared using slightly modified method reported by Weerakkody *et al*. (2010). Samples after removal of skin were subjected into slicing then dried overnight at 40°C followed by grinding using mixer for 3 min to make fine particles. In this study two types of solvents, ethanol (food grade) and water were used for the extraction of samples. Ethanol extracts were obtained by adding 10 g of ground sample into 100 ml of 90 % ethanol and agitated for 24 h at 28°C in a rotary shaker. Rotary evaporator was used to evaporate the filtrate to dryness under vacuum. Water extraction

was prepared as described for ethanol extraction but the solvent was replaced with sterile distilled water. Finally ethanol and water extracts were diluted with appropriate volume of 90% ethanol and sterile distilled water respectively for 50% concentration. All of the extracts were kept at 4°C prior to antimicrobial test.

Antimicrobial susceptibility test

The antimicrobial susceptibility test was performed using disc diffusion test (Norhana *et al.*, 2009; Weerakkody *et al*., 2010). An overnight bacterial culture was suspended into 5 ml of 0.1% saline and then adjusted with 0.5 Mc Farland standards. A 50 µl of the bacterial suspension was spread well with sterile swabs on Mueller Hinton (MH) media. Sterile paper discs (Whatman No. 1, 5.5 mm diameter) were impregnated with 10 µl of each extract and then dried in laminar flow prior to use. Positive control was prepared by subjecting 10 µl of 20 mg/ml Streptomycin (Fluka, Switzerland) on paper disc for the susceptibility test on the same plate. The discs were transferred by using sterile forceps onto Muller Hilton media which were previously seeded with bacterial suspension. All essays were performed in triplicates. The plates were incubated for 24 hours at 37°C on upright position. Diameter of clear zone surrounding each disc marked as diameter of inhibition zone (DIZ) in millimetre. Inhibition zones with diameter > 16 mm were considered highly active. Diameters in the range of 12 to 16 mm were considered moderately active and those < 12 mm were considered as no antimicrobial activity.

Statistical analysis

The significance of differences was determined by one-way analysis of variance (ANOVA) of the Minitab statistical software, version 14 (Minitab Inc., State College, PA, USA) and differences with P values of < 0.05 were considered statistically significant.

Results and Discussion

Ethanol, water and juice extracts of musk lime, key lime and lemon were screened for their antibacterial activity using disc diffusion test. The pH value of juice extracts were within the range of 2.31-2.39. It is quite similar to pH value of sour lime as reported by Fletcher *et al.* (2008). The pH value of key lime juice is significantly ($p < 0.05$) lower than that of musk lime and lemon juice extracts. Nevertheless, the antimicrobial activity is not merely influenced by the acidity of the fruit juice extract (Friedman *et al.*, 2004). Extracts were applied at concentration level of 100 (pure extract) and 50% for the antimicrobial activity. The results are presented in Table 1, Table 2 and Table 3. Ethanol, water and juice extracts revealed various range of DIZ against all the tested bacteria. It is well known that the DIZ depends on extrinsic and intrinsic factors. The extrinsic parameters classified as size of bacterial suspension, volume of well or size of the disc impregnated with extract, temperature and duration of the incubation and pH of the medium (Jayana *et al.*, 2010). Moreover, type of extraction solvent, extracted forms, harvesting periods and geographical basis may lead to variation in the antimicrobial properties of spices/herbs (Nanasombat and Lohasupthawee, 2005; Hoque *et al.*, 2007). In this study, type of extraction solvent and different concentration level were the prime factor for the various range of DIZ revealed by musk lime, key lime and lemon extracts.

Ethanol extracts of musk lime, key lime and lemon exhibited significantly ($p < 0.05$) higher inhibitory activity at concentration level of 100% compared to water and juice extracts at similar concentration level. Low antimicrobial activity of aqueous and juice extracts might be due to lack of the solubility of bioactive compounds in aqueous and juice extracts (Parekh and Chanda, 2007). Superior effect of ethanol extracts may be attributed to its greater dissolving power than water (Aboaba *et al.*, 2006). However, least antimicrobial activity of ethanol extracts (100%) of key lime, lemon and musk lime were observed towards *Salmonella typhimurium*, *Salmonella typhi* and *Salmonella typhimurium* respectively. These bacteria are classified as gram negative food borne pathogens which are less susceptible towards spice/herb extracts than gram positive bacteria. This is due to the variation in the multilayered thin cell wall of peptidoglycan and an outer lipopolysaccharide membrane of gram negative bacteria, while gram positive bacteria consist of a thick monolayer of peptidoglycan cell wall Moreover, enzymes present in perisplasmic part of gram negative bacteria may rupture and deactivate active compounds (spice/herb extract) that penetrate from external membrane. This phenomenon may lead to lower sensitivity of *S. typhimurium*, *S. typhi* and other gram negative bacteria tested toward spice/herb extracts (Parekh and Chanda, 2007; Weerakkody *et al*., 2010; Keskin and Toroglu, 2011). Parekh and Chanda (2007) mentioned *S. typhimurium* as the least susceptible bacteria as none of the extract tested gave positive result. Among the food borne pathogens tested, *S. aureus* was most susceptible to ethanol, water and juice extracts of musk lime, key lime and lemons at concentration level of 100 and 50%. Dupont *et al.* (2006) also found that *S. aureus* was strongly

Table 1. Inhibitory activity (inhibition zone; mm) of 50 and 100% ethanol, water and juice extracts of musk lime against food related bacteria

Microorganism	Streptomycin 20 mg/ml	Lime		
100%		Ethanol	Water	Juice
Food borne pathogens				
Salmonella typhimurium	10.4 ± 1.7	11.7 ± 1.2^{c}	8.0 ± 0.0^{b}	0.0 ± 0.0^{a}
Salmonella typhi	8.8 ± 1.3	13.0 ± 1.7^{c}	8.0 ± 0.0^{b}	0.0 ± 0.0^{a}
Staphylococcus aureus	29.6 ± 0.9	20.7 ± 1.2^{c}	13.7 ± 0.6^{b}	10.7 ± 1.2^{a}
E. coli	23.0 ± 1.6	17.3 ± 0.6^{c}	9.7 ± 0.6^{b}	0.0 ± 0.0^{a}
Listeria monocytogenes	25.0 ± 1.7	15.3 ± 1.2^{b}	11.7 ± 1.2^{b}	12.3 ± 0.6^{a}
Spoilage bacteria				
Aeromonas hydrophila-like DNA group 2	20.0 ± 1.3	18.7 ± 0.6^{c}	11.7 ± 0.6^{b}	0.0 ± 0.0^{a}
A. hydrophila	20.6 ± 1.3	19.7 ± 1.2^{c}	13.0 ± 0.0^{b}	9.0 ± 1.0^{a}
A. media-like DNA group 5B	20.7 ± 1.4	17.3 ± 1.5^{b}	9.0 ± 0.0^{a}	7.0 ± 0.0^{a}
A. sobria DNA group 7	20.8 ± 1.9	24.0 ± 1.0^{c}	15.3 ± 0.6^{b}	9.7 ± 0.6^{a}
A. sobria DNA group 8	21.9 ± 1.9	24.3 ± 1.2^{c}	15.0 ± 0.0^{b}	7.7 ± 0.6^{a}
A. veronii	18.3 ± 1.7	39.7 ± 1.5^{c}	25.3 ± 1.2^{b}	0.0 ± 0.0^{a}
A. veronii DNA group 10	20.2 ± 0.8	24.0 ± 0.0^{c}	16.3 ± 0.6^{b}	9.3 ± 1.5^{a}
Pseudomonas fluorescens	11.3 ± 1.5	31.7 ± 0.6^{b}	12.7 ± 1.5^{a}	10.0 ± 1.0^{a}
Pseudomonas lundensis	11.4 ± 1.5	27.7 ± 1..5^{c}	17.3 ± 1.5^{b}	12.7 ± 1.2^{a}
Carnobacterium maltaromaticum	23.0 ± 1.9	29.0 ± 0.0^{b}	14.3 ± 1.5^{a}	12.7 ± 1.5^{a}
Yersenia ruckeri	9.9 ± 1.3	14.7 ± 1.5^{b}	8.3 ± 1.2^{a}	7.7 ± 1.2^{a}
Psychrotrophic immobilis	28.7 ± 1.7	26.7 ± 1.2^{c}	14.7 ± 1.5^{b}	0.0 ± 0.0^{a}
50%				
Food borne pathogens				
Salmonella typhimurium	10.4 ± 1.7	10.7 ± 1.2^{c}	6.7 ± 0.6^{b}	0.0 ± 0.0^{a}
Salmonella typhi	8.8 ± 1.3	9.0 ± 1.0^{c}	6.7 ± 0.6^{b}	0.0 ± 0.0^{a}
Staphylococcus aureus	29.6 ± 0.9	13.0 ± 1.7^{c}	7.7 ± 1.5^{b}	0.0 ± 0.0^{a}
E. coli	23.0 ± 1.6	10.0 ± 1.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{a}
Listeria monocytogenes	25.0 ± 1.7	12.0 ± 1.7^{b}	7.3 ± 1.2^{b}	0.0 ± 0.0^{a}
Spoilage bacteria				
Aeromonas hydrophila-like DNA group 2	20.0 ± 1.3	16.7 ± 1.2^{c}	11.0 ± 1.0^{b}	0.0 ± 0.0^{a}
A. hydrophila	20.6 ± 1.3	15.3 ± 0.6^{c}	10.0 ± 0.0^{b}	0.0 ± 0.0^{a}
A. media-like DNA group 5B	20.7 ± 1.4	12.7 ± 1.5^{c}	7.7 ± 1.5^{b}	0.0 ± 0.0^{a}
A. sobria DNA group 7	20.8 ± 1.9	18.3 ± 0.6^{c}	10.3 ± 1.2^{b}	6.0 ± 0.0^{a}
A. sobria DNA group 8	21.9 ± 1.9	16.7 ± 1.5^{b}	7.3 ± 0.6^{b}	6.3 ± 0.6^{a}
A. veronii	18.3 ± 1.7	30.3 ± 1.5^{c}	16.3 ± 0.6^{b}	0.0 ± 0.0^{a}
A. veronii DNA group 10	20.2 ± 0.8	20.0 ± 0.0^{c}	9.7 ± 0.6^{b}	6.7 ± 0.6^{a}
Pseudomonas fluorescens	11.3 ± 1.5	26.0 ± 1.0^{c}	11.3 ± 0.6^{b}	9.7 ± 0.6^{a}
Pseudomonas lundensis	11.4 ± 1.5	18.0 ± 1.0^{c}	10.3 ± 0.6^{b}	0.0 ± 0.0^{a}
Carnobacterium maltaromaticum	23.0 ± 1.9	14.7 ± 1.5^{b}	6.7 ± 1.2^{b}	7.0 ± 0.0^{a}
Yersenia ruckeri	9.9 ± 1.3	14.3 ± 1.2^{c}	7.3 ± 0.6^{b}	0.0 ± 0.0^{a}
Psychrotrophic immobilis	28.7 ± 1.7	23.0 ± 1.7^{c}	14.0 ± 1.0^{b}	0.0 ± 0.0^{a}

[A] Values are mean ± S.D (mm), with rows different letters are significantly ($p < 0.05$) different. Data represents a means of 3 replicates.
[B] DIZ value 0 mm means the extract had no inhibition against bacterium.
[C] Positive control: Streptomycin 20 mg/ml.

inhibited by water and ethanol extracts of Australian native herbs.

Water extract of musk lime, key lime and lemon at concentration level of 100% showed the largest DIZ against *Aeromonas veronii* (25.3 mm), *Pseudomonas lundensis* (20.0 mm) and *A. veronii* (25.3 mm) correspondingly. Largest DIZ of juice extracts of musk lime, key lime and lemon were observed against *P. lundensis* (12.7 mm) (and *C. maltaromaticum* 12.7 mm), *A. veronii* (23.3 mm) and *A. veronii* (20.0 mm) respectively. Noticeably, key lime juice extracts revealed the greatest antimicrobial activity. It might be owing to the significant pH value of key lime juice (2.31 ± 0.01a) compared to musk lime (2.38 ± 0.02b) and lemon juice (2.39 ± 0.01b) extracts. Conversely, juice extracts of all the samples especially at concentration level of 50% exhibited the least or no inhibitory activity against all the tested microorganisms. This is with the exception for lemon juice extract whereby moderate level inhibition has

Table 2. Inhibitory activity (inhibition zone; mm) of 50 & 100% ethanol, water and juice extracts of key lime against food related bacteria

Microorganism	Streptomycin 20 mg/ml	Key lime		
100%		Ethanol	Water	Juice
Food borne pathogens				
Salmonella typhimurium	10.4 ± 1.7	11.0 ± 1.0^{b}	6.7 ± 0.6^{a}	7.7 ± 0.6^{a}
Salmonella typhi	8.8 ± 1.3	9.3 ± 0.6^{b}	9.7 ± 0.6^{b}	8.0 ± 0.0^{a}
Staphylococcus aureus	29.6 ± 0.9	16.3 ± 0.6^{a}	15.7 ± 1.5^{a}	14.7 ± 1.5^{a}
E. coli	23.0 ± 1.6	11.7 ± 0.6^{c}	14.6 ± 0.6^{b}	8.0 ± 0.0^{a}
Listeria monocytogenes	25.0 ± 1.7	13.0 ± 0.0^{b}	11.7 ± 1.2^{ab}	10.0 ± 0.0^{a}
Spoilage bacteria				
Aeromonas hydrophila-like DNA group 2	20.0 ± 1.3	18.3 ± 1.2^{c}	11.7 ± 0.6^{b}	13.3 ± 1.5^{a}
A. hydrophila	20.6 ± 1.3	19.7 ± 0.6^{b}	12.3 ± 0.6^{a}	9.7 ± 0.6^{a}
A. media-like DNA group 5B	20.7 ± 1.4	14.7 ± 0.6^{a}	12.3 ± 1.5^{a}	13.3 ± 1.5^{a}
A. sobria DNA group 7	20.8 ± 1.9	21.0 ± 1.0^{c}	11.0 ± 0.0^{b}	14.0 ± 1.0^{a}
A. sobria DNA group 8	21.9 ± 1.9	18.3 ± 1.5^{b}	17.0 ± 1.0^{b}	12.0 ± 1.0^{a}
A. veronii	18.3 ± 1.7	26.7 ± 1.5^{b}	15.7 ± 0.6^{a}	23.3 ± 1.5^{b}
A. veronii DNA group 10	20.2 ± 0.8	22.0 ± 1.0^{b}	20.3 ± 0.6^{b}	12.0 ± 1.7^{a}
Pseudomonas fluorescens	11.3 ± 1.5	27.0 ± 1.0^{c}	15.3 ± 1.2^{b}	8.0 ± 0.0^{a}
Pseudomonas lundensis	11.4 ± 1.5	18.7 ± 1.5^{b}	20.0 ± 1.0^{b}	14.0 ± 0.0^{a}
Carnobacterium maltaromaticum	23.0 ± 1.9	19.3 ± 1.2^{c}	20.0 ± 0.0^{b}	10.0 ± 0.0^{a}
Yersenia ruckeri	9.9 ± 1.3	12.3 ± 0.6^{a}	10.7 ± 1.5^{a}	13.7 ± 0.6^{a}
Psychrotrophic immobilis	28.7 ± 1.7	20.3 ± 1.2^{b}	12.0 ± 1.0^{a}	13.7 ± 1.2^{a}
50%				
Food borne pathogens				
Salmonella typhimurium	10.4 ± 1.7	8.7 ± 0.6^{c}	0^{a}	6.0 ± 0.1^{b}
Salmonella typhi	8.8 ± 1.3	8.0 ± 1.0^{b}	8.7 ± 0.6^{b}	6.0 ± 0.0^{a}
Staphylococcus aureus	29.6 ± 0.9	11.3 ± 1.5^{a}	12.7 ± 1.5^{a}	8.7 ± 1.5^{a}
E. coli	23.0 ± 1.6	7.3 ± 0.6^{b}	10.7 ± 1.2^{c}	0^{a}
Listeria monocytogenes	25.0 ± 1.7	11.0 ± 1.0^{b}	8.7 ± 1.5^{ab}	8.0 ± 0.0^{a}
Spoilage bacteria				
Aeromonas hydrophila-like DNA group 2	20.0 ± 1.3	20.7 ± 1.5^{c}	11.3 ± 0.6^{b}	8.3 ± 0.6^{a}
A. hydrophila	20.6 ± 1.3	11.7 ± 1.2^{b}	8.0 ± 1.7^{a}	6.0 ± 0.0^{a}
A. media-like DNA group 5B	20.7 ± 1.4	11.7 ± 1.5^{a}	10.0 ± 1.0^{a}	8.7 ± 1.2^{a}
A. sobria DNA group 7	20.8 ± 1.9	20.7 ± 0.6^{c}	10.0 ± 0.0^{b}	8.0 ± 0.0^{a}
A. sobria DNA group 8	21.9 ± 1.9	15.0 ± 2.0^{b}	14.0 ± 1.0^{b}	6.3 ± 0.6^{a}
A. veronii	18.3 ± 1.7	26.0 ± 2.0^{c}	14.0 ± 0.0^{b}	10.0 ± 0.0^{a}
A. veronii DNA group 10	20.2 ± 0.8	17.3 ± 1.2^{c}	13.7 ± 1.5^{b}	10.0 ± 0.0^{a}
Pseudomonas fluorescens	11.3 ± 1.5	24.7 ± 1.5^{c}	10.0 ± 1.0^{b}	7.0 ± 0.0^{a}
Pseudomonas lundensis	11.4 ± 1.5	15.7 ± 1.2^{b}	15.0 ± 1.0^{b}	10.0 ± 0.0^{a}
Carnobacterium maltaromaticum	23.0 ± 1.9	10.7 ± 1.2^{a}	14.7 ± 1.2^{a}	6.0 ± 0.0^{a}
Yersenia ruckeri	9.9 ± 1.3	11.0 ± 1.0^{b}	12.0 ± 1.0^{a}	10.0 ± 0.0^{a}
Psychrotrophic immobilis	28.7 ± 1.7	18.3 ± 1.5^{c}	7.7 ± 0.6^{b}	7.0 ± 0.0^{a}

[A] Values are mean ± S.D (mm), with rows different letters are significantly ($p < 0.05$) different. Data represents a means of 3 replicates.
[B] DIZ value 0 mm means the extract had no inhibition against bacterium.
[C] Positive control: Streptomycin 20 mg/ml.

been achieved against *A. veronii* (13.0 mm) and *Psychrotrophic immobilis* (13.3 mm). Aibinu *et al*. (2007) reported type of extraction solvent as a principal factor for extraction of active compounds from lime fruits.

Type of extraction solvent was concerned as a factor for the various range of DIZ for each sample in this study. DIZ for ethanol, water and juice extracts of musk lime at concentration level of 100% were in the range of 11.7-39.0 mm, 8.0-25.3 mm and 0-12.7 mm respectively (Table 2). Ethanol and water extracts of musk lime was most effective against *A. veronii* (39.0 mm and 25.3 mm), while its the juice extract was most effective against *P. lundensis* (12.7 mm), *C. maltaromaticum* (12.7 mm) and *Listeria monocytogenes* (12.3 mm). The results revealed for 50% concentration were in the range of 9.0-30.3 mm, 0-16.3 mm and 0-9.7 mm correspondingly for ethanol, water and juice extracts of musk lime. The results obviously proved that ethanol extract at 100% concentration has the wide range of DIZ in contrast to water and juice extracts. Both 100 and 50% concentrated juice extract showed least or no activity towards all of the microorganisms tested. The results clearly demonstrate that ethanol is the suitable extraction solvent for musk lime. Noticeably, DIZ for ethanol extract at 50% concentration basis is more prominent than 100% water and juice extracts' antimicrobial effectiveness. Successive extraction of bioactive compound and antimicrobial

Table 3. Inhibitory activity (inhibition zone; mm) of 50 & 100% ethanol, water and juice extracts of lemon against food related bacteria

Microorganism	Streptomycin 20 mg/ml	Lemon		
100%		Ethanol	Water	Juice
Food borne pathogens				
Salmonella typhimurium	10.4 ± 1.7	9.0 ± 1.0^{c}	11.7 ± 1.2^{b}	0^{a}
Salmonella typhi	8.8 ± 1.3	11.3 ± 1.2^{b}	8.0 ± 0.0	7.3 ± 0.6^{a}
Staphylococcus aureus	29.6 ± 0.9	22.3 ± 1.5^{c}	17.7 ± 1.5^{b}	12.3 ± 0.6^{a}
E. coli	23.0 ± 1.6	13.3 ± 0.6^{b}	13.0 ± 1.0^{b}	0^{a}
Listeria monocytogenes	25.0 ± 1.7	19.0 ± 1.0^{c}	14.7 ± 1.2^{b}	7.0 ± 0.0^{a}
Spoilage bacteria				
Aeromonas hydrophila-like DNA group 2	20.0 ± 1.3	22.3 ± 1.5^{c}	17.7 ± 0.6^{b}	9.3 ± 0.6^{a}
A. hydrophila	20.6 ± 1.3	20.3 ± 0.6^{c}	14.3 ± 0.6^{b}	8.0 ± 1.0^{a}
A. media-like DNA group 5B	20.7 ± 1.4	21.7 ± 2.1^{c}	15.0 ± 1.0^{b}	10.3 ± 1.2^{a}
A. sobria DNA group 7	20.8 ± 1.9	24.3 ± 0.6^{c}	17.0 ± 0.0^{b}	8.3 ± 0.6^{a}
A. sobria DNA group 8	21.9 ± 1.9	23.7 ± 1.5^{c}	18.3 ± 1.5^{b}	10.0 ± 1.7^{a}
A. veronii	18.3 ± 1.7	32.0 ± 1.7^{c}	25.3 ± 1.5^{b}	20.0 ± 1.0^{a}
A. veronii DNA group 10	20.2 ± 0.8	20.0 ± 0.0^{b}	19.7 ± 0.6^{b}	9.3 ± 1.2^{a}
Pseudomonas fluorescens	11.3 ± 1.5	29.0 ± 1.0^{c}	23.7 ± 1.5^{b}	9.0 ± 1.0^{a}
Pseudomonas lundensis	11.4 ± 1.5	26.0 ± 1.7^{b}	18.0 ± 1.7^{a}	17.0 ± 0.0^{a}
Carnobacterium maltaromaticum	23.0 ± 1.9	32.7 ± 1.2^{c}	18.7 ± 1.2^{b}	10.0 ± 0.0^{a}
Yersenia ruckeri	9.9 ± 1.3	18.7 ± 1.2^{c}	12.7 ± 1.5^{b}	9.3 ± 0.6^{a}
Psychrotrophic immobilis	28.7 ± 1.7	25.0 ± 1.0^{b}	17.3 ± 0.6^{a}	19.3 ± 0.6^{a}
50%				
Food borne pathogens				
Salmonella typhimurium	10.4 ± 1.7	8.7 ± 1.2^{b}	8.7 ± 1.5^{b}	0^{a}
Salmonella typhi	8.8 ± 1.3	10.7 ± 0.6^{c}	7.7 ± 0.6^{b}	0^{a}
Staphylococcus aureus	29.6 ± 0.9	21.0 ± 1.7^{c}	12.0 ± 1.0^{b}	5.3 ± 0.6^{a}
E. coli	23.0 ± 1.6	11.3 ± 0.6^{c}	9.0 ± 1.0^{b}	0^{a}
Listeria monocytogenes	25.0 ± 1.7	18.0 ± 1.0^{c}	12.3 ± 1.2^{b}	6.0 ± 0.0^{a}
Spoilage bacteria				
Aeromonas hydrophila-like DNA group 2	20.0 ± 1.3	20.3 ± 0.6^{c}	13.3 ± 1.2^{b}	6.3 ± 0.6^{a}
A. hydrophila	20.6 ± 1.3	19.0 ± 0.0^{c}	13.7 ± 0.6^{b}	0^{a}
A. media-like DNA group 5B	20.7 ± 1.4	15.0 ± 1.0^{c}	9.0 ± 1.0^{b}	0^{a}
A. sobria DNA group 7	20.8 ± 1.9	21.7 ± 0.6^{c}	12.7 ± 0.6^{b}	6.3 ± 0.6^{a}
A. sobria DNA group 8	21.9 ± 1.9	23.7 ± 0.6^{c}	14.3 ± 1.5^{b}	7.3 ± 0.6^{a}
A. veronii	18.3 ± 1.7	31.7 ± 0.6^{c}	21.3 ± 0.6^{b}	13.0 ± 1.0^{a}
A. veronii DNA group 10	20.2 ± 0.8	15.0 ± 0.0^{c}	11.7 ± 0.6^{b}	6.3 ± 0.6^{a}
Pseudomonas fluorescens	11.3 ± 1.5	21.0 ± 1.0^{c}	14.0 ± 1.0^{b}	7.0 ± 0.0^{a}
Pseudomonas lundensis	11.4 ± 1.5	23.7 ± 0.6^{b}	10.3 ± 1.5^{a}	11.0 ± 1.0^{a}
Carnobacterium maltaromaticum	23.0 ± 1.9	21.3 ± 0.6^{c}	13.7 ± 1.2^{b}	0^{a}
Yersenia ruckeri	9.9 ± 1.3	13.3 ± 1.5^{c}	7.0 ± 1.0^{b}	0^{a}
Psychrotrophic immobilis	28.7 ± 1.7	20.3 ± 0.6^{b}	11.0 ± 1.0^{a}	13.3 ± 1.5^{a}

[A] Values are mean ± S.D (mm), with rows different letters are significantly ($p < 0.05$) different. Data represents a means of 3 replicates.
[B] DIZ value 0 mm means the extract had no inhibition against bacterium.
[C] Positive control: Streptomycin 20 mg/ml.

activity of herbs/ spices is discriminatory and solvent dependent (Parekh and Chanda, 2007). According to Weerakkody *et al*. (2010) chemical components or active compounds that will be obtained are related to the extraction technique. Ethanol extracts of *Phyllanthus acidus* demonstrated greatest inhibitory activity for the tested microorganisms (Jagessar *et al.*, 2008). Moreover, lime is consists of exclusive flavonoid components which lead to its antioxidant, antibiotic and anti cancer properties. The primary organic acid found in lime juice is citric acid (Jayana *et al.*, 2010). This organic acid might contributed to the prominent antimicrobial results of ethanol extracts of musk lime at 100 and 50% concentration level.

Ethanol, water and juice extracts of key lime showed various significant DIZ for all the tested microorganisms. The range of DIZ for ethanol, water and juice extracts of key lime is 9.3-27.0 mm, 6.7-20.3 mm and 7.7-23.3 mm (Table 2) respectively for 100% concentrated extract. The largest DIZ obtained for 100% ethanol extract was against *Pseudomonas fluorescens* (27.0 mm). Water extract (100%) showed greatest DIZ against *A. veronii* DNA group 10 (20.3 mm) which is significantly different than juice extract but not with ethanol extract. Juice extracts revealed greatest inhibitory activity against *A. veronii* with DIZ of 23.3 mm. 100% ethanol, water and juice extracts of key lime did not exhibit significant difference towards *S. aureus*, *A. media*-like DNA group 5B and *Yersenia ruckeri.* Similarly, 50% extracts showed

no significant inhibitory activity for *S. aureus*, *A. media*-like DNA group 5B and *C. maltaromaticum*. Noticeably, 50% key lime juice extract showed no or least inhibitory activity against all the tested microorganisms.

Antimicrobial activity exhibited by 100% ethanol, water and juice extracts of lemon were in the range of 9.0-32.7 mm, 8.0-25.3 mm and 0-20.0 mm correspondingly (Table 3). Largest significant DIZ exhibited by 100% ethanol extracts of lemon was against *C. maltaromaticum* (32.7 mm) and *A. veronii* (32.0 mm). As for 100% water and juice extracts broadest activity observed on *A. veronii* with 23.7 mm and 20.0 mm correspondingly. Ethanol extract (100%) was significantly active towards all microorganisms tested except *S. typhimurium* and *S. typhi*. This could be attributed to the clarification mentioned earlier. Moreover, *S. typhimurium* has been stated as largely resistant bacteria as none of the plant extract tested could inhibit this bacteria (Parekh and Chanda, 2007). Lemon juice (100%) extracts revealed high DIZ for *A. veronii* (20.0 mm), *P. lundensis* (17.0 mm) and *Psychrotrophic immobilis* (19.3 mm). 50% lemon juice extracts revealed no inhibitory activity towards all the tested bacteria except for moderate activity against *P. immobilis* (13.3 mm) and *A. veronii* (13.0 mm). Both 50% ethanol and water extracts demonstrated high DIZ agaisnt *A. veronii* (Adedeji *et al.*, 2007) have reported that the crude aqueous extracts of lime and lemon revealed strong antibacterial activity towards certain strains of microorganisms.

Conclusion

Generally, ethanol extracts of musk lime, key lime and lemon at 100% concentration level exhibited higher antimicrobial activity towards all the tested microorganisms compared to water and juice extracts. Greater effect of ethanol extracts may be attributed to its greater dissolving power than water. Diameter of inhibition zone (DIZ) for ethanol extracts of musk lime, key lime and lemon were in the range of 11.7-39.0 mm, 9.3-27.0 mm and 9.0-32.7 mm respectively. Ethanol extracts of musk lime, key lime and lemon revealed strongest antimicrobial activity against food spoilage bacteria, Aeromonas veronii and Pseudomonas fluorescens. Noticeably, the results reveal the significance of musk lime, key lime and lemon extracts to control spoilage bacteria and certain food borne pathogens which lead hazard to human health. However, further studies are required to evaluate the active constituents causative to the antimicrobial action in various solvent (ethanol, water and juice) extracts of musk lime, lemon, and key lime. The prominent antimicrobial activity from musk lime, key lime and lemon extracts may attribute them as natural food preservatives depend on the precise problem to be tackled as well in pharmaceuticals field to treat infectious diseases.

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