International Food Research Journal 23(3): 1262-1267 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

In vitro antimicrobial activity of *Cymbopogon citratus* (lemongrass) extracts against selected foodborne pathogens

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Article history

Received: 7 July 2015
Received in revised form: 16 September 2015
Accepted: 30 September 2015

Keywords

Antimicrobial activity
Cymbopogon citratus
Foodborne pathogen
Lemongrass
Natural food preservative

Abstract

Microbial contamination in food system poses risk towards public health. The usage of synthetic and chemical preservatives to prevent the contamination has become a growing concern due to the presence of deleterious and harmful substances that can cause environment and health problems in prolonged exposure. Thus, there are needs to overcome this problem by using natural products as food preservatives. In this study, the antimicrobial activities of methanolic *Cymbopogon citratus* (lemongrass) extracts were tested against five foodborne pathogens, namely *Bacillus cereus, Escherichia coli* O157:H7, *Klebsiella pneumoniae, Staphylococcus aureus* and *Candida albicans*. The susceptibility test, minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) were conducted using the broth microdilution techniques as described by Clinical and Laboratory Standard Institute (CLSI). *C. citratus* extract showed antimicrobial activity against all tested foodborne pathogens; *B. cereus, E. coli* O157:H7, *K. pneumoniae, S. aureus* and *C. albicans* with the inhibition zone of 12 mm, 7.5 mm, 11 mm, 10 mm and 9 mm, respectively. The MIC of *C. citratus* extract against *B. cereus, E. coli* O157:H7, *K. pneumoniae, S. aureus* and *C. albicans* was 0.08 mg/ml, 0.63 mg/ml, 0.04 mg/ml, 0.31 mg/ml, and 0.16 mg/ml, respectively, while the MBC or MFC was 1.25 mg/ml, 2.50 mg/ml, 2.50 mg/ml, 1.25 mg/ml and 1.25 mg/ml, respectively. Time–kill curves were determined to assess the correlation between MIC and bactericidal activity of *C. citratus* extract at concentrations ranging from 0× MIC to 4× MIC. The bactericidal endpoint for *B. cereus, E. coli* O157:H7, *S. aureus and C.* albicans was at 4× MIC after 2 h, 4× MIC after 2 h, 4× MIC after 30 min and 4× MIC after 4 h, respectively whereas *K. pneumoniae* was not completely killed after 4 hours of incubation at 4× MIC. The potent antimicrobial activity of *C. citratus* extract may support its usage as natural antimicrobial agent

Introduction

Microbial safety of food in terms of food contamination and spoilage by microorganisms has always been a concern to consumers and food industries. The implementation of preservation techniques in food system to prolong shelf life of food and control the growth of food-borne pathogens such as thermal treatment, water activity reduction, and synthetic antimicrobial agents has been associated with some drawbacks including changes in organoleptic characterization, nutrient loss and safety issues of using chemical additives (Negi, 2012). In addition, the frequent usages of antibiotics on food borne pathogens have led to the emergence of antibiotic-resistant microorganisms (Sánchez *et al*., 2010; Negi, 2012). The development of microbial resistance renders the common antimicrobial agents ineffective that leads to exploitation of other antimicrobial substances from other sources (Shin and Lim, 2004). Therefore, other alternatives of developing novel antimicrobial agent from natural sources, such as traditional medicinal plants, spices and herbs need to be explored (Rukayadi *et al.*, 2008; Sánchez *et al*., 2010).

In Malaysia, there are several possible candidates for plant with antimicrobial activities and one of them is *Cymbopogon citratus* or commonly known as lemongrass. It is used widely as an essential ingredient in Asian cuisines due to the sharp lemon flavour. *C. citratus*, which belongs to the family of *Gramineae,* is commonly used in folk medicine for treatment of nervous and gastrointestinal disturbances (Bassolé *et al*., 2011). It is also used as antispasmodic, analgesic, anti-inflammatory, anti-pyretic, diuretic and sedative (de F. Melo *et al.*, 2001; Bassolé *et al.*, 2011;

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Francisco *et al.*, 2011). Francisco *et al.* (2011) also mentioned that *C. citratus* leaves extract has potent antioxidant activity due to its polyphenolic content and is a potential source of new anti-inflammatory drug. It was reported that *C. citratus* to have antibacterial, antifungal, antitumoral, anticancer and insecticide activities (Negrelle and Gomes, 2007). The antimicrobial activity of *C. citratus* against a series of microorganisms is due to the abundance of citral and essential oil components i.e Geranial, Myrcene, 6-Methylhept-5-en-22-one (Negrelle and Gomes, 2007; Calo *et al*., 2015). This led to suggestion that *C. citratus* may have antimicrobial activities against *Bacillus cereus, Escherichia coli* O157:H7, *Klebsiella pneumoniae, Staphylococcus aureus* and *Candida albicans.*

Therefore, the objective of this study is to evaluate the antimicrobial activity of *Cymbopogon citratus* (lemongrass) extract against the aforementioned food borne pathogens. The susceptibility of *C. citratus* extract in term of minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC / MFC) will be determined. Time–kill curves will also be conducted to assess the correlation between MIC and bactericidal activity of *C. citratus* extract at different concentrations, ranging from 0× MIC to 4× MIC.

Materials and Methods

Plant material

Cymbopogon citratus was collected from Pasar Borong Selangor, Malaysia before deposited in Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia until further usage.

Preparation of Cymbopogon citratus *extract*

Extraction of *C. citratus* was done according to Rukayadi *et al*. (2008), with slight modification. A 100 g of dried *C. citratus* was grounded and extracted twice with 400 ml of 100% (v/v) methanol for 1 week at room temperature. The plant extract was filtered with Whatman filter paper No. 2 (Whatman International Ltd., Middlesex, England) and concentrated with a rotary vacuum evaporator (Heidolph VV2011, Schwabach, Germany) at 50˚C, yielding methanolic extract. The methanolic extract was dissolved in 10% aqueous dimethylsulfoxide (DMSO) to obtain 100 mg/ml and the solution was further diluted to obtain 10 mg/ml stock solutions. A 10% DMSO did not kill microorganisms that being tested in this study.

Inoculum preparation

Bacillus cereus ATCC 10987, *Escherichia coli* O157:H7 ATCC 25922, *Klebsiella pneumoniae* ATCC 15692, *Candida albicans* ATCC 10231 were obtained from the American Type Culture Collection (Rockville, MD, USA). *Staphylococcus aureus* KCCM 11764 was obtained from Korean Culture Center of Microorganisms (Seoul, South Korea).

B. cereus, E. coli O157:H7, *K. pneumoniae* and *S. aureus* were grown on Mueller Hinton agar (MHA) (Difco, Franklin Lakes, NJ, USA), while C. albicans on Sabouraud Dextrose agar (SDA) (Difco, Franklin Lakes, NJ, USA), aerobically for 24 hours at 37˚C, whereas inoculum cell suspension was prepared by propagating a single colony of each microbial species in 10 ml of Mueller Hinton broth (MHB) or Sabouraud Dextrose broth (SDB) at 37˚C overnight with 200 rpm agitation. A 1 µl of microbial suspension was further diluted into the ratio of 1:10 (microbial suspension: MHB/SDB) to yield inoculum with 10^7 – 10^8 CFU/ml which was then compared with 0.5 Mc Farland.

In vitro *susceptibility test using disc-diffusion method*

Methanolic extract of *Cymbopogon citratus* was screened for antimicrobial activity using the standard paper disc diffusion assay as described by Clinical and Laboratory Standards Institute (CLSI, 2003). The bacterial strains and *C. albicans* were streaked on MHA and SDA plates, respectively, with sterile cotton swabs. Sterile filter paper discs, 6 mm diameter, were loaded with 10 µl of 10 mg/ml (w/v) *C. citratus* extract. A 1 mg/ml of chlorhexidine (CHX) used as positive control while 10% DMSO as negative control. The plates were incubated at 37˚C for 12 - 24 hours. Evidence of clear zone (including the disc diameter) was measured in millimeter (mm) unit.

Determination of minimum inhibitory concentrations (MIC) and minimum bactericidal or fungicidal concentration (MBC / MFC)

In vitro tests were performed in a 96-well microtiter plate to determine the MIC and MBC of *C. citratus* extract against *B. cereus, E. coli* O157:H7, *K. pneumoniae, S. aureus* and *C. albicans* using standard broth microdilution methods (CLSI, 2003) with an inoculum (10^7 – 10^8 CFU/ml). Briefly, a two-fold dilution of *C. citratus* extract stock solution was mixed with the microorganisms in MHB or SDB. Column 12 of the microtiter plate contained the highest concentration of the extract, while column 3 contained the lowest concentration. Column 2 served as the positive control for all samples (MHB or SDB,

with inoculums), and column 1 as the negative control (MHB or SDB, without inoculum and antimicrobial agent). Microtiter plates were incubated aerobically at 37˚C for 24 hours. The MIC was defined as the lowest concentration of antimicrobial agent that resulted in the complete inhibition of visible growth.

MBC or MFC were determined for each microbial species by transferring the media from each well of microtiter plate showing no visible growth, and sub-culturing onto MHA plates. The plates were incubated at 37˚C for 24 hours until growth was seen at positive control. MBC was defined as the corresponding concentrations required killing microorganisms completely.

Determination of time-kill curve

Time–kill assay was performed on each bacterial species in MHB medium and *Candida* strain in SDB medium according to Rukayadi *et al*. (2006) with modification. The *C. citratus* extract was diluted with the MHB or SDB medium containing prepared inoculum to obtain final concentrations of 0× MIC, 1× MIC, 2× MIC and 4× MIC for each microbial species. Cultures (1 ml of final volume) were incubated at 37˚C with 200 rpm agitation. At pre-determined time of 0, 0.5, 1, 2, and 4 hours, 100 µl aliquots were transferred into Eppendorf tubes and was serially diluted with 990 µl of 1% phosphate buffered saline (PBS). Then, 20 µl was pipetted and spread onto MHA or SDA plates and incubated at 37˚C for 24 hours. The number of colonies appeared on the plates was counted and the number of colonies was determined and reported as CFU/ml. Assays were carried out in duplicate.

Results and Discussion

Cymbopogon citratus active compounds are known to possess various biological activities. It has been proven to have bactericidal, fungicidal, anti-oxidant, anti-inflammatory, antihypertensive, antinociceptive, anti-obesity, and anxiolytic (Tzotzakis and Economakis, 2007; Moore-Neibel *et al*., 2012; Olorunnisola *et al.*, 2014). The antimicrobial activity of *C. citratus* extracts were evaluated against five species of foodborne pathogens namely *B. cereus* ATCC 10987, *E.coli* O157: H7 ATCC 25922, *K. pneumoniae* ATCC 15692, *S. aureus* KCCM 11764, and *C. albicans* ATCC 10231. Results analysed based on the inhibition zone, minimum inhibitory concentration (MIC) and minimum bactericidal / fungicidal concentration (MBC / MFC).

In disc diffusion test, the presence of clear zone around the paper disc is denoted as the inhibition of growth of the microorganism (Jun *et al*., 2013). Table 1 shows the potentiality of *C. citratus* to inhibit all tested foodborne pathogens with different level of susceptibility based on the diameter of clear zone (mm); the wider the diameter of the clear zone means more susceptible or have a higher susceptibility. Bacteria species; *B. cereus* (12.00 mm), *K. pneumoniae* (11.00 mm), *S. aureus* (10.00 mm) have higher susceptibility compared to fungal species; *C. albicans* (9.00 mm) except for *E. coli* O157:H7 (7.50 mm). It could be due to the resistance of the *E. coli* towards the antimicrobial components in *C. citratus* extracts. *E. coli* O157:H7 is a Gram-negative bacterium, which had an outer layer on the cell wall that is made up of lipopolysaccharides. Therefore, the cell is protected under the lipopolysaccharide layer and is more resistant to the antimicrobial agents (Bin *et al.*, 2007).

Table 1. Inhibition zone, minimum inhibitory concentration, and minimum bactericidal or fungicidal concentration of *Cymbopogon citratus* extract against food borne pathogens

Susceptibility Test / Microorganism	Inhibition zone (mm)	MIC (mg/ml)	MBC / MFC (mg/ml)
Bacillus cereus	12.00 ± 1.41	0.08	1.25
Escherichia coli O157:H7	7.50 ± 0.71	0.63	2.50
Klebsiella pneumonia	11.00 ± 1.41	0.04	2.50
Staphylococcus aureus	10.00 ± 0.00	0.31	1.25
Candida albicans	9.00 ± 1.41	0.16	1.25

MIC is defined as the minimum concentration of the antimicrobial needed to inhibit at least 99% of visible growth of the microorganisms, whereas MBC or MFC is the minimum of the antimicrobials needed to kill at least 99% of growth. Table 1 summarizes the MIC, MBC or MFC value of each of the microorganisms. *E. coli* O157:H7 has the strongest resistance to the antimicrobials as the MIC and MBC needed by *C. citrus* extracts was highest; 0.63 mg/ml and 2.50 mg/ml, respectively. On the other hand, *K. pneumoniae* was found to be easy to get inhibited (MIC: 0.04 mg/ml) but extremely difficult to be killed (MBC: 2.50 mg/ml). It shows that *K. pneumoniae* possesses a specific self-protective mechanism to protect them from being killed after they got inhibited. Zainol *et al.*, (2003) discussed that there

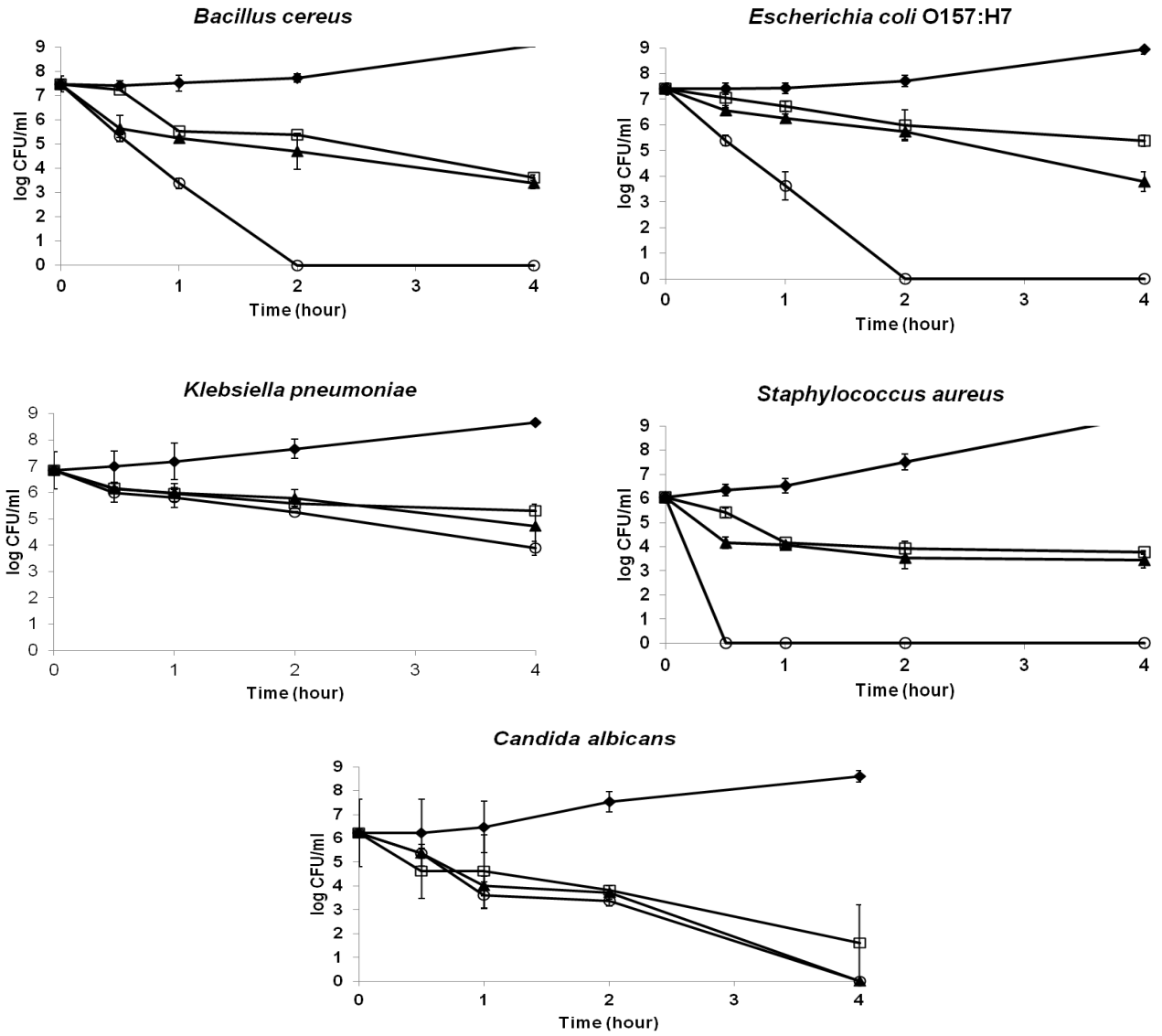

Figure 1. Representative time–kill plots for the food borne pathogens following exposure to *Cymbopogon citratus* extract at 0× MIC (♦), 1× MIC (□), 2× MIC (▲) and 4× MIC (○) after endpoint (4 h).
(a) *Bacillus cereus* (0, 0.08, 0.16 and 0.32 mg/ml)
(b) *Escherichia col*i O157:H7 (0, 0.63, 1.25 and 2.50 mg/ml)
(c) *Klebsiella pneumoniae* (0, 0.04, 0.08 and 0.16 mg/ml)
(d) *Staphylococcus aureus* (0, 0.31, 0.63 and 1.25 mg/ml)
(e) *Candida albicans* (0, 0.16, 0.31 and 0.63 mg/ml)

are some species which are easier to get inhibited but hard to be killed due to their adaptive ability.

Figure 1(a) to 1(e) showed the time-kill curve for each species of tested foodborne pathogens. The bactericidal endpoints for *B. cereus, E. coli* O157:H7, *S. aureus* and *C. albicans* were reached at 4× MIC after 2 hours, 4× MIC after 2 hours, 4× MIC after 30 minutes and 4× MIC after 4 hours of incubation periods, respectively. While, no bactericidal endpoints was found for *K. pneumoniae,* but the growth were decreased until 1 log, 2 log and 3 log CFU/ml reduction after being treated with *C. citratus* extract for 4 hours at 1× MIC, 2× MIC and 4× MIC, respectively. The reduction of *B. cereus, E. coli* O157:H7, *S. aureus* and *C. albicans* in the CFU/ml were ≥ 3 log units (99.9%) at *C. citratus* concentration of 2× MIC, 2× MIC, 4× MIC and 1× MIC, respectively. The strongest bacteriostatic and fungiostatic activity was found on *B. cereus* and *C. albicans* which were at 0.16 mg/ml at 2× MIC and 1× MIC, respectively. These data demonstrated that the killing activity was dependent on the concentration of *C. citratus* extract and the types of microorganisms tested.

According to Bhoj *et al.* (2011) and Adegbegi *et al*. (2012), *C. citratus* contains active components like alkaloids, tannis, flavanoids, terpenes and phenolic compounds. Phenols and flavonoids

are widely been reported can caused membrane disruption while alkaloids are thought to inhibit the growth of microorganisms by affecting their genetic materials (Cowan, 1999). Besides that, the essential oils in many plant extracts was also reported to possess hydrophobic characteristic that enable them to partition in the lipid component of bacterial membrane, rendering them permeable and leading to leakage of the cell contents (Burt, 2004; Vimol *et al*., 2012).

Conclusions

In conclusion, the potential of *Cymbopogon citratus* extract to be used as natural antimicrobials agent is recommendable as antimicrobial activity against *Bacillus cereus, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli* O157:H7 and *Candida albicans* were demonstrated. Further study on the active constituents and possible inhibitory mechanisms of *C. citratus* extract would be interesting. The development of natural plant extracts and active compounds would be a great alternative of food preservatives to the food industries.

Acknowledgements

This work was supported by RUGS initiative 4 (RUGS 4) – UPM to Yaya Rukayadi under the project number: 02-05-11-1586RU (2011).

References

Adegbegi, A.J., Usunobun, U. and Adewumi, B.L. 2012. Comparative studies on the chemical composition and antimicrobial activities of the ethanolic extracts of lemongrass leaves and stems. Asian Journal of Medical Sciences 4(4): 145-148.

Alzarokey, N.S. and Nakahara, K. 2003. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology 80(3): 223-230.

Aqil, F., Ahmad, I. and Owais, M. 2006. Evaluation of anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity and synergy of some bioactive plant extracts. Biotechnology Journal 1(10): 1093-1102.

Bassole, I.H.N., Lamien-Meda, A., Bayala, B., Obame, L.C., Ilboudo, A.J., Franz, C. and Dicko, M.H. 2011. Chemical composition and antimicrobial activity of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils alone and in combination. Phytomedicine 18(12): 1070-1074.

Bhoj, R.S., Vidya, S., Raj, K.S. and Ebibeni, N. 2011. Antimicrobial activity of lemongrass *(Cymbopogon citratus)* oil against microbes of environmental, clinical and food origin. International Research of Pharmacy and Pharmacology 1(9): 228-236.

Bin, S., Yi-Zhong, C., John, D.B. and Harold, C. 2007. The *in vitro* antibacterial activity of dietary spice and medical herb extracts. International Journal of Food Microbiology 117: 112-119.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology 94(3): 223-253.

Calo, J. R., Crandall, P. G., O'Bryan, C. A. and Ricke, S. C. 2015. Essential oils as antimicrobials in food system. Food Control 54: 111-119

Clinical and Laboratory Standards Institute (CLSI). 2003. Reference method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A6. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.

Cowan, M. M. 1999. Plant products as antimicrobial agents. Clinical Microbiology Review 12: 564-582.

De F. Melo, S., Soares, S.F., da Costa, R.F., da Silva, C.R., de Oliveira, M.B.N., Bezerra, R.J.A.C. and Bernardo-Filho, M. 2001. Effect of the *Cymbopogon citratus, Maytenus ilicifolia* and *Baccharis genistelloides* extracts against the stannous chloride oxidative damage in Escherichia coli. Mutation Research/ Genetic Toxicology and Environmental Mutagenesis 496(1-2): 33-38.

Francisco, V., Figueirinha, A., Neves, B.M., Garcia-Rodriguez, C., Lopes, M.C., Cruz, M.T. and Batista, M.T. 2011. *Cymbopogon citratus* as a source of new and safe anti-inflammatory drugs: bio-guided assay using lipopolysaccharide-stimulated macrophages. Journal of Ethnopharmacology 133(2): 818-827.

Jun, H., Kim, J., Bang, J., Kim, H., Beuchat, L.R. and Ryu, J.H. 2013. Combined effects of plant extracts in inhibiting the growth of *Bacillus cereus* in reconstituted infant rice cereal. International Journal of Food Microbiology 160(3): 260-266.

Moore-Neibel, K., Gerber, C., Patel, J., Friedman, M. and Ravishankar, S. 2012. Antimicrobial activity of lemongrass oil against *Salmonella enterica* on organic leafy greens. Journal of Applied Microbiology 112(3): 485–492.

Negi, P.S. 2012. Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. International Journal of Food Microbiology 156(1): 7-17.

Negrelle, R.R.B. and Gomes, E.C. 2007. *Cymbopogon citratus* (DC) Stapf: chemical composition and biological activities. Revista Brasileira de Plantas Medicinais 9: 80-92.

Olorunnisola, S.K., Asiyanbi, H. T., Hammed, A. M. and Simsek, S. 2014. Biological properties of lemongrass: An overview. International Food Research Journal 21(2): 455-462.

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

Rukayadi, Y., Shim, J. S. and Hwang, J. K. 2006. In vitro anticandidal activity of xanthorrhizol isolated from

Curcuma xanthorrhiza Roxb. Journal of Antimicrobial Chemotherapy 57(6): 1231-1234.

Sanchez, E., Garcia, S. and Heredia, N. 2010. Extracts of edible and medicinal plant damage membranes of *Vibrio cholerae.* Applied and Environmental Microbiology 76(20): 6888-6894.

Shin, S. and Lim, S. 2004. Antifungal effects of herbal essential oils alone and in combination with ketoconazole against *Trichophyton* spp. Journal of Applied Microbiology 97(6): 1289-1296

Tzortzakis, N. G. and Economakis, C. D. 2007. Antifungal activity of lemongrass (*Cympopogon citratus* L.) essential oil against key postharvest pathogens. Innovative Food Science and Emerging Technologies 8(2): 253–258.

Vimol, S., Chanwit, T., Veena, N., Nuntayan, B. and Sompom, S. 2012. Antibacterial activity of essential oils from *Citrus hystrix* (makrut lime) against respiratory tract pathogens. Science Asia 38: 212-217.

Zainol, M.I., Yusoff, K.M. and Yusof, M.Y.M. 2013. Antibacterial activity of selected Malaysian honey. BMC Complementary and Alternative Medicine 13(129): 1-10.