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Original Article

Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms

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Abstract

Ethyl acetate extracts and hydrodistillated-essential oils from peels of *Citrus* spp. were investigated for their antimicrobial activities against food related microorganisms by broth microdilution assay. Overall, ethyl acetate extracts from all citrus peels showed stronger antimicrobial activities than their essential oils obtained from hydrodistillation. The ethyl acetate extract of kaffir lime (*Citrus hystrix* DC.) peel showed broad spectrum of inhibition against all Gram-positive bacteria, yeast and molds including *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Saccharomyces cerevisiae* var. *sake* and *Aspergillus fumigatus* TISTR 3180. It exhibited minimum inhibitory concentration (MIC) values of 0.28 and 0.56 mg/ml against *Sac. cerevisiae* var. *sake* and *B. cereus*, respectively while the minimum bactericidal concentration (MBC) values against both microbes were 0.56 mg/ml. The MIC values of the extract against *L. monocytogenes*, *A. fumigatus* TISTR 3180 and *S. aureus* were 1.13 mg/ml while the MBC values against *L. monocytogenes* as well as *A. fumigatus* TISTR 3180 and *S. aureus* were 2.25 and 1.13 mg/ml, respectively. The major components of the ethyl acetate extract from kaffir lime were limonene (31.64 %), citronellal (25.96 %) and β-pinene (6.83 %) whereas β-pinene (30.48 %), sabinene (22.75 %) and citronellal (15.66 %) appeared to be major compounds of the essential oil obtained from hydrodistillation.

Keywords: antimicrobial activity, *Citrus hystrix* DC., essential oils, food related microorganisms, hydrodistillation

1. Introduction

Consumer demand for natural preservatives has increased, whereas the safety aspect of chemical additives has been questioned. Essential oils and extracts obtained from many plants have recently gained a great popularity and scientific interest. The antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, cinnamon, garlic and onion against food-related microorganisms as well as their applications in food system have been investigated and reviewed (Holley and Patel, 2005; Burt, 2004; Gill *et al*., 2002). Moreover, essential oils from many medicinal plants were also exhibited antimicrobial activity against many pathogenic microbes (Melendez and Capriles, 2006; Samy, 2005; Wannissorn *et al*., 2005). Phenolic compounds present in essential oils have been recognized as the bioactive components for the antimicrobial activity. Most plant phenolic compounds are classified as Generally Recognized as Safe (GRAS) substances, therefore they could be used to prevent growth of many food-borne and food spoilage microorganisms in foods.

Citrus fruits belong to six genera (*Fortunella*, *Eremocitrus*, *Clymendia*, *Poncirus*, *Microcitrus* and *Citrus*), which are native to the tropical and subtropical regions of Asia, but the major commercial fruits belong to genus *Citrus*. The genus *Citrus* includes several important fruits such as oranges, mandarins, lime, lemons and grape fruits. Citrus

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essential oils are present in fruit flavedo in great quantities. This layer consists of the epidermis covering the exocarp consisting of irregular parenchymatous cells, which are completely enclosing numerous glands or oil sacs. Citrus essential oils are a mixture of volatile compounds and mainly consisted of monoterpene hydrocarbons (Sawamura *et al*., 2004). Citrus oils are mixtures of over a hundred compounds that can be approximated into three fractions: terpene hydrocarbons, oxygenated compounds and non-volatile compounds. The terpene fraction can constitute from 50 to more than 95% of the oil; however, it makes little contribution to the flavor and fragrance of the oil.

It is well known that essential oils from *Citrus* spp. have pronounced antimicrobial effect against both bacteria and fungi (Lanciotti *et al*., 2004; Caccioni *et al*., 1998; Dabbah *et al*., 1970). Citrus essential oils could represent good candidates to improve the shelf life and the safety of minimally processed fruits (Lanciotti *et al*., 2004), skim milk and low-fat milk (Dabbah *et al*., 1970). However, most studies have focused on essential oils from subtropical citrus. The essential oils from two cultivars of tropical citrus, including *Citrus hystrix* DC., and *Citrus aurantifolia* exhibited antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhi* (Chaisawadi *et al*., 2003). In this study, antibacterial as well as antifungal activities of essential oils and ethyl acetate extracts from various tropical citrus cultivars available in Thailand were compared and evaluated against both food-borne and food spoilage microorganisms. Each group of food-related microorganisms, including Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*), Gram-negative bacteria (*Salmonella* sp. and *Escherichia coli* O157: H7 DMST 12743), spore-forming bacteria (*Bacillus cereus*), yeast (*Saccharomyces cerevisiae* var. *sake*) and mold (*Aspergillus fumigatus* TISTR 3180) were selected as the test microorganisms. Additionally, the chemical components of the extract exhibiting high antimicrobial activity were also determined.

2. Materials and Methods

2.1 Plant materials

Fruits of seven citrus cultivars of "kaffir lime or ma-krut" (*Citrus hystrix* DC.), "lime or ma-nao" (*Citrus aurantifolia* Swingle), "round kumquat or som-jeed" (*Citrus japonica* Thunb), "neck-orange or som-juk" (*Citrus reticulate* Blanco), "chugun" (*Citrus reticulate* cv. Chugun), "pomelo" (*Citrus maxima* Merr.) and "acidless orange or som-tra or som-cheng" (*Citrus paradisi*) were collected at the mature stage from fruit orchards around Songkhla area during May to July, 2005.

2.2 Extraction procedures

Citrus peels (500 g) were subjected to hydrodistillation for 4 hour to obtain essential oil. The essential oils were dried over anhydrous sodium sulfate and stored under N_2 in sealed vials at 4°C. Ethyl acetate extracts were obtained by grinding 500 g of citrus peels to fine powder, then soaking in 2 liter of ethyl acetate and shaking at the speed of 130 rpm for 8 h. The citrus peel residue was removed by filtration through filter paper No.4 (Whatman). The solvent extracts were dried over anhydrous sodium sulfate. Ethyl acetate was removed by rotary-vacuum evaporator, and removed completely by nitrogen evaporator to yield dry ethyl acetate extracts, which were stored under N_2 in sealed vials at 4°C. Yields of essential oils and ethyl acetate extract obtained were calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered}}{\text{Weight of fresh citrus peel}} \times 100$$

2.3 Microorganisms and their growth conditions

Microbial strains including *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* sp., *Saccharomyces cerevisiae* var. *sake* and *Aspergillus fumigatus* TISTR 3180 were obtained from culture collection of Microbiology Laboratory, Faculty of Agro-Industry, Prince of Songkhla University. *Escherichia coli* O157: H7 DMST 12743 was obtained from the Department of Medical Science (DMSC), Ministry of Health (Bangkok, Thailand). All bacterial and yeast strains were cultivated in Mueller Hinton Broth (MHB) and Yeast Malt Broth (YMB), respectively at 37°C for 18 h. Approximately 1 ml of culture was transferred to 9 ml of broth medium and incubated at 37°C for another 15 h, cell concentration was then adjusted to obtain final concentration of 10^6 CFU/ml using MHB and YMB for bacteria and yeast, respectively. Fungal spores were prepared by growing mold on Potato Dextrose Agar (PDA) at 37°C for 7 days, and spores were suspended in sterile 1% tween-80. Spore count was performed by using hemacytometer, and adjusted to obtain 10^6 spores/ ml with Potato Dextrose Broth (PDB).

2.4 Evaluation of antimicrobial activity

All hydrodistilled-essential oils and ethyl acetate extracts of citrus peels from seven citrus cultivars were tested for antimicrobial activity against foodborne pathogens by broth microdilution assay. Twenty microliter of microbial suspension (cultured and diluted as mentioned above to yield 10^6 CFU/ml or spores/ml) was added to 180 microliter of medium broth to yield a final concentration of 10^5 CFU/ml in each well. The extracts were added at the two-fold dilution manner, ranging from 0.07 to 2.25 mg/ml (dry mass of crude extract or essential oil), in a 96-well microtiterplate, which was incubated at 37°C. The microbial growth was determined at 24 h of incubation by measuring the absorption at 600 nm. The lowest concentration of crude citrus extract/hydrodistillated oils required to completely inhibit

microbial growth (no change of OD_{600}) after incubation at 37°C for 24 hours (for bacteria) or 48 hours (for yeast and mold) was reported as minimum inhibitory concentration (MIC). Microbial viability of test microorganisms in the culture broth, which showed no microbial growth (OD_{600}) was determined by transferring and spreading the treated culture broth on agar plate, then incubated at 37°C. The lowest concentration of ethyl acetate extract or hydrodistillated-essential oil required to completely destroy test microorganisms (no growth on the agar plate) after incubation at 37°C for 24 hours (for bacteria) or 48 hours (for yeast and mold) was reported as minimum bactericidal concentration for bacteria (MBC) or minimum fungicidal concentration (MFC) for yeast and mold. Each ethyl acetate extract or essential oil was tested for its antimicrobial activity in triplication on two separate runs (NCCLS, 1994).

2.5 Analysis of chemical composition

The analysis of the essential oil was performed on Gas Chromatography-Mass Spectrophotometry (GC-MS) on a Hewlett Packard 5890 Gas Chromatography with on (30 m x 0.25 mm i.d., 0.25 mm) Rtx-5MS column and a Hewlett Packard 5972 mass selective detector. For MS detection, an electron ionization system with ionization energy of 70 eV with MS transfer line at temperature of 300°C was used. Column temperature was initially kept at 70°C for 2 min, and gradually increased at the rate of 4°C per min to 220°C, at which the temperature was held for 5 min and finally raised to 300°C at 10°C per min. Helium was used as carrier gas at a flow rate of 1 ml/ min. The sample of 1 ml was injected in the acquisition mode. The components were identified based on the comparison of their relative retention times and mass spectra with those of Wiley 275.L library data of the GC/MS system. (Agnihotri *et al*., 2004)

3. Results and Discussion

3.1 Effects of citrus cultivars and extraction procedures on production yields of the extracts

The extraction yields of hydrodistillated-essential oils and ethyl acetate extracts from fresh peels of *Citrus* spp. widely varied depending on citrus cultivars. For each cultivar, the production yields of the hydrodistillated-essential oils were much lower than that from extraction with ethyl acetate. The production yields of hydrodistillated-essential oils and ethyl acetate extracts from all citrus cultivars are given in Figure 1. Ethyl acetate extraction of kaffir lime, lime, pomelo, acidless orange, neck orange, chogun and round kumquat peels provided the production yields of 2.56, 1.73, 1.57, 0.88, 2.44, 2.06 and 1.11%, whereas only 0.95, 0.57, 0.24, 0.2, 0.79, 0.69 and 0.28% yields, respectively were obtained from hydrodistillation. Kaffir lime peel yielded the highest amount of ethyl acetate extract and hydrodistillated essential oil comparing to other citrus cultivars. The lowest

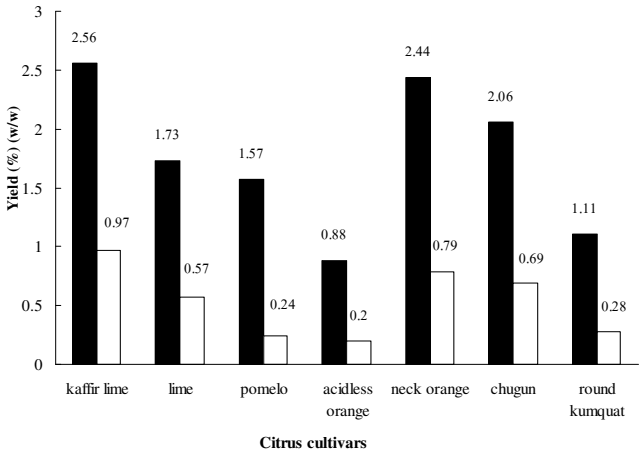

Figure 1. Yields of ethyl acetate extracts (■) and essential oils (□) from peels of various citrus cultivars.

yields of both extract and essential oil were obtained from acidless orange peel.

3.2 Antimicrobial activity of citrus extracts

Antimicrobial activities against pathogenic *E. coli* and *S. aureus* of ethyl acetate extracts from fresh peels and dried peels of tropical citrus fruits including lime, kaffir lime and pomelo peels were compared. The extracts from dried peels had lost some antimicrobial activity (Table 1), although the drying process was performed at low temperature of 55°C. All extracts from both fresh and dried lime, kaffir lime and pomelo peels had antibacterial activity against *S. aureus*, but the ones from fresh peels showed higher inhibition (wider inhibitory zones). The extracts from fresh lime and kaffir lime peels inhibited growth of *E. coli* whereas no activity was observed with the ones from dried peels, indicating loss of certain inhibitory components during drying process, particularly volatile compounds.

Antimicrobial activities from hydrodistillated-essential oils and ethyl acetate extracts from fresh citrus peels were performed against various food-related microorganisms by broth microdilution assay. The ethyl acetate extracts showed stronger antimicrobial activity than the ones obtained from hydrodistillation. Particularly, the one from kaffir lime peel which showed broad spectrum inhibitory against all Gram-positive bacteria (Table 2), yeast and mold (Table 3) tested. It exhibited MIC values of 0.28 and 0.56 mg/ml against *Sac. cerevisiae* var. *sake* and *Bacillus cereus*, respectively, while its MFC or MBC values for both microbes were 0.56 mg/ml. The MIC values of the ethyl acetate extract against *L. monocytogenes*, *A. fumigatus* TISTR 3180 and *S. aureus* were 1.13 mg/ml, while the MBC or MFC values for *L. monocytogenes*, *A. fumigatus* TISTR 3180 were 2.25 mg/ml and the value for *S. aureus* was 1.13 mg/ml. However, all Gram-negative tested including *Salmonella* sp. and *E. coli* O157: H7 were resistant to all citrus extracts at the concentration tested (Table 4). Gram-positive bacteria were more sensitive

Table 1. Antimicrobial activity of ethyl acetate extracts from fresh and dried (55°C, 48 h) citrus peels. The activity was determined by disk diffusion assay, and diameters of the inhibition zones were measured and expressed as millimeters.

Citrus peels	Concentration (µg)	*Staphylococcus aureus*			*Escherichia coli*		
		Lime	Kaffir lime	Pomelo	Lime	Kaffir lime	Pomelo
Fresh peels	200	12.0	12.0	12.0	10.5	9.5	NI*
	100	9.5	11.0	8.0	9.0	8.0	NI
	50	10.5	9.5	NI	5.0	NI	NI
	25	10.0	10.0	NI	NI	NI	NI
Dried peels	200	8.5	8.0	NI	NI	NI	NI
	100	7.5	7.0	NI	NI	NI	NI
	50	7.5	7.0	NI	NI	NI	NI
	25	6.0	8.0	NI	NI	NI	NI

NI = No Inhibition

Table 2. MIC and MBC (mg/ml) of crude extracts from *Citrus* spp. prepared by ethyl acetate extraction and hydrodistillation against Gram-positive bacteria

Microorganisms	Extraction methods	MIC & MBC	Concentration (mg/ml)						
			Kaffir lime	Lime	Pomelo	Acidless orange	Chugun orange	Neck kumquat	Round
B. cereus	Ethyl acetate	MIC	0.56	0.56	>2.25	>2.25	2.25	2.25	2.25
		MBC	0.56	0.56	>2.25	>2.25	2.25	2.25	2.25
	Hydrodistillation	MIC	1.13	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
		MBC	1.13	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
S. aureus	Ethyl acetate	MIC	1.13	1.13	>2.25	>2.25	>2.25	>2.25	>2.25
		MBC	1.13	1.13	>2.25	>2.25	>2.25	>2.25	>2.25
	Hydrodistillation	MIC	2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
		MBC	2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
L. monocytogenes	Ethyl acetate	MIC	1.13	1.13	>2.25	>2.25	>2.25	0.56	>2.25
		MBC	2.25	2.25	>2.25	>2.25	>2.25	1.13	>2.25
	Hydrodistillation	MIC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
		MBC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25

to essential oils than Gram-negative bacteria due to their outer membrane barriers (Burt, 2004). Among Gram-positive bacteria *B. cereus* was the most sensitive to the ethyl acetate extract from kaffir lime peel (MIC 0.56 mg/ml) whereas *L. monocytogenes* was the most resistant (MIC 1.13 mg/ml).

Similarly, the ethyl acetate extract from lime peel (*Citrus aurantifolia* Swingle) showed broad spectrum inhibitory against all Gram-positive bacteria, yeast and mold tested. However, the ethyl acetate extract from kaffir lime peel was more effective than the ethyl acetate extract from lime peel against *Sac. cerevisiae* var. *sake* and *A. fumigatus* TISTR3180. Both extracts exhibited MIC and MBC values against *B. cereus* at 0.56 mg/ml and against *S. aureus, and L. monocytogenes* at 1.13 mg/ml. The results were correlated to Chaisawadi *et al*. (2003) reported that *Citrus hystrix* DC., and *Citrus aurantifolia* displayed antibacterial activities against *B. cereus* and *S. aureus*. Interestingly, ethyl acetate extracts from acidless orange (*Citrus paradisi*), chugun (*Citrus reticulate* cv. Chugun) and pomelo (*Citrus maxima* Merr.) specifically inhibited *A. fumigatus* TISTR 3180 with MIC values of 0.28, 0.56 and 0.56 mg/ml and MFC values of 0.28, 0.56 and 1.13 mg/ml, respectively. However, these particular extracts showed low inhibitory activity against the rest of the microbes tested, except the extracts from pomelo and chugun, which were also inhibitory activity against *Sac. cerevisiae* var. *sake* with MIC values of 0.56 and 1.13 mg/ml and MFC values of 0.56 and 1.13, respectively. Many studies reported pronounced antifungal activity as well as

Table 3. MIC and MFC (mg/ml) of crude extracts from *Citrus* spp. prepared by ethyl acetate extraction and hydrodistillation against yeast and mold.

Microorganisms	Extraction methods	MIC & MBC	Concentration (mg/ml)						
			Kaffir lime	Lime	Pomelo	Acidless orange	Chugun orange	Neck kumquat	Round
Sac. cerevisiae var. *sake*	Ethyl acetate	MIC	0.28	0.56	0.56	>2.25	1.13	1.13	2.25
		MFC	0.56	0.56	0.56	>2.25	1.13	1.13	2.25
	Hydrodistillation	MIC	0.28	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
		MFC	0.56	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
A. fumigatus TISTR 3180	Ethyl acetate	MIC	1.13	2.25	0.56	0.28	0.56	2.25	>2.25
		MFC	2.25	2.25	1.13	0.28	0.56	>2.25	>2.25
	Hydrodistillation	MIC	2.25	>2.25	>2.25	>2.25	>2.25	2.25	>2.25
		MFC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25

Table 4. MIC and MBC (mg/ml) of crude extracts from *Citrus* spp. prepared by ethyl acetate extraction and hydrodistillation against Gram-negative bacteria

Microorganisms	Extraction methods	MIC & MBC	Concentration (mg/ml)						
			Kaffir lime	Lime	Pomelo	Acidless orange	Chugun orange	Neck kumquat	Round
Salmonella sp.	Ethyl acetate	MIC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
		MBC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
	Hydrodistillation	MIC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
		MBC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
E. coli O157: H7 DMST 12743	Ethyl acetate	MIC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
		MBC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
	Hydrodistillation	MIC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25
		MBC	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25	>2.25

antibacterial activity of essential oils from various citrus cultivars grown in temperate climate. Antifungal substances of hydrodistilled-essential oils from orange (*Citrus sinensis* cv. "Washington navel", "Sanguinello", "Tarocco", "Moro", "Valencia late", and "Ovale"), bitter (sour) orange (*C. aurantium*), mandarin (*C. deliciosa* cv. "Avana"), grapefruit (*C. paradisi* cv. "Marsh seedless" and "Red Blush"), citrange (*C. sinensis x Poncirus trifoliata* cv. "Carrizo" and "Troyer"), and lemon (*C. limon* cv. "Femminello" were reported to be inhibitory against *Penicillium digitatum* and *Penicillium italicum* (Caccioni *et al*., 1998). Industrial citrus essences from sweet orange, red orangeade, bitter orange, red orange, sicily orange, sweet lime and red orangeade had shown inhibitory activity against *Sac. cerevisiae* (Belletti *et al*., 2004). Essential oil from *Citrus sinensis* (L.) inhibited growth of *A. niger* (Shamar and Tripathi, *in press*). In addition, the antibacterial activity of essential oils from lemon was reported against various Gram-positive and Gram-negative bacteria (Baratta *et al*., 1998).

3.3 Chemical compositions of kaffir lime essential oil and ethyl acetate extract

Citrus oils were a mixture of volatile compounds and consisted mainly of monoterpene hydrocarbons (Sawamura *et al*., 2004). The composition of kaffir lime essential oil and ethyl acetate extract were, therefore, analyzed by GC/MS system, and identification of component was based on retention times, computer matching with Wiley 275.L data library, comparison of the fragmentation pattern with those reported in the literature and conjunction with authentic sample in case of major components. The major constituents of ethyl acetate extract from kaffir lime peel were limonene (31.64%), citronellal (25.99%) and β-pinene (6.83%), whereas β-pinene (30.48%), sabinene (22.75%) and citronellal (15.66%) appeared to be major components of the hydrodistillated-essential oil (Table 5). Manosroi *et al*. (1999) reported that the essential oil of kaffir lime peels was the mixture of many compounds such as β-pinene (30.6 %), limonene (29.2%),

Table 5. Chemical composition of ethyl acetate extract and hydrodistilled-essential oil from kaffir lime

Components	Kaffir lime peel	
	Ethyl acetate extract	hydrodistilled-essential oil
limonene	31.64	8.13
citronellal	25.96	15.67
beta-pinene	6.83	30.48
sabinene	5.43	22.75
citronellol	1.89	3.24
citronellyl acetate	5.41	-
delta-cadinene	3.21	-
alpha-copaene	2.99	-
trans-caryophyllene	2.88	-
l-isopulegol	2.13	-
trans-sabinene hydrate	1.74	-
germacrene D	1.34	-
myrcene	1.33	-
4-terpineol	-	6.61
alpha-pinene	-	3.05
m-cymene	-	0.85

sabinene (22.6 %) and citronellal (4.2 %), which was cor-related to this study. However, the variation of each component amount depended on several parameters including ripeness of fruits, vegetative stage of plant, storage condition and extraction method (Lota *et al*., 2000). Several studies have also shown that monoterpenes exert microbial membrane damaging effects (Sikkema *et al*., 1995; Cox *et al*., 2000). A positive correlation between monoterpenes other than limonene and sesquiterpene content of the oils and the pathogenic fungi inhibition was observed and reported (Caccioni *et al*., 1998).

The higher antibacterial activity against Gram-positive bacteria of the ethyl acetate extract than the hydrodistillated-essential oil from kaffir lime peel was related to citronellal content which was also higher in the ethyl acetate extract. Therefore citronellal could be a potential component, which contributed to the antibacterial activity. However, antifungal activity against *Sac. cerevisiae* of the hydrodistillated-essential oil may caused by another components particularly β-pinene and sabinene, which were present at the high concentration. The antimicrobial activity of kaffir lime peel may be contributed by many bioactive components, each of which may affect different groups of microorganisms, hence the broad spectrum inhibition of the extract.

4. Conclusion

Hydrodistillated-essential oils from all citrus cultivars had less inhibitory activity, compared to the ethyl acetate extracts. The ethyl acetate extract from kaffir lime showed broad spectrum inhibitory activity against Gram-negative bacteria, Gram-positive bacteria, yeast and mold tested. However, all Gram-negative bacteria tested including *Salmonella* sp., and pathogenic *E. coli* O157: H7 DMST 12743 were more resistant to all citrus extracts. Among Gram-positive bacteria tested, *Bacillus cereus* was the most sensitive (MIC 0.56 mg/ml), whereas *L. monocytogenes* was the most resistant (MIC 1.13 mg/ml). The major constituents of the ethyl acetate extract from kaffir lime were limonene, citronellal and β-pinene whereas β-pinene, sabinene and citronellal appeared to be major compounds of the essential oil obtained from hydrodistillation. These components may contribute to the broad spectrum antimicrobial activity of the kaffir lime extract. However, further evaluation performed with the pure compounds is required for the definite conclusion of the bioactive compounds contributing to the antimicrobial activity of the kaffir lime peel extracts.

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