

EFFECT OF ESSENTIAL OIL FROM *Feroniella lucida* (SCHEFF.) SWINGLE FRUIT ON *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a bacterium commonly found on human skin and mucus membrane linked to the severity of the chronic inflammatory skin disease, and a contaminant in food. This study presented the effect of essential oil extracted from *Feroniella lucida* (Scheff.) Swingle fruit for its antibacterial activity against two strains of *Staphylococcus aureus*, ATCC 29213 and TISTR 517; and its chemical composition. The hydro-distillated essential oil yield from the peel was approximately 0.72% (v/w, fresh weight), compared to the average yield of 0.10% (v/w) from the plant leaves. The major components of the peel oil were decyl acetate (56.20% of the total GC-MC peak area), decanal (22.49%), and dodecyl acetate (6.81%). The oil could inhibit both strains, but the ATCC 29213 was more sensitive than TISTR 517 with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 0.125 and 2.0, and 0.5 and 16.0 mg/mL, respectively. For the mode of action performed for the strain ATCC 29213, the essential oil had a significant effect to inhibit the bacterium growth within 2 h, and caused protein, potassium ion, and adenosine triphosphates (ATP) leakages, but it had no effect on the leakage of nucleic acids. Under scanning electron microscope (SEM) observation of the essential oil effect on the bacterial cell morphology, the crease and furrow of ATCC 29213 cells were detected. These results encourage further application of the oil from the edible fruit as anti-*Staphylococcus aureus* to develop skin care products as well as to be used in food industry.

Keywords: Essential oil, *Feroniella lucida* (Scheff.) Swingle, Antibacterial activity, *Staphylococcus aureus*, Mode of action

Introduction

Staphylococcus aureus is a species of *Staphylococcus* bacteria commonly distributing in nature and are general found on skin and mucus membrane of human and warm-blooded animals, and widely contaminating in environment and especially in food (Irlinger, 2008). The bacterium is one of the main bacteria widely present in various food, and easily contaminated during food process leading to food spoilage and foodborne illness (Montanari *et al.*, 2015), and recognized

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as a commensal and pathogen bacterium able to colonize the human skin surface, which may subsequently lead to infection. *Staphylococcus aureus* can cause severe skin and soft tissue infections (Olaniyi *et al.*, 2017). It is also linked to the severity and worsening of the chronic inflammatory skin disease (Totte *et al.*, 2016).

Natural plant components are the leading choice for safe antimicrobial agents to control or inactivate the pathogens or spoilage bacteria in both food and human (Fisher and Phillips, 2006; Tajkarimi *et al.*, 2010). Essential oils, the natural metabolites produced by organisms especially plant, are volatile and complex compounds with strong flavour and scent. Aromatic plants established essential oil as secondary metabolites which may protect the plants as antibacterial, antivirals, antifungals, insecticides (Bakkali *et al.*, 2008). Essential oils composed of twenty to sixty mixture chemical components and may differ even the same species because of a vary in quality, quantity and in composition according to climate, soil composition, plant organ, age, and vegetative cycle stage (Angioni *et al.*, 2006; Masotti *et al.*, 2003). The major group of essential oil compositions was reported to contain terpenes, terpenoids, aromatic, aliphatic, and also derivative of the constituents, all characterized by low molecular weight (Bakkali *et al.*, 2008). Essential oils become the most useful chemical preservatives used to control and inactivate foodborne, and plant and animal pathogens. The *Nandina domestica* Thunb. oil could be used as a potential alternative to synthetic preservatives (Bajpai *et al.*, 2008). The *Eucalyptus globulus* fruit essential oil exhibited an interesting antibacterial activity against pathogenic and spoilage microorganisms, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are known for their resistance to the antibiotics (Bey-Ould Si Said *et al.*, 2016). Australian tea tree essential oil has been reported.

To inhibit *Escherichia coli*, the animal pathogen (Hayes *et al.*, 1997). The *in vitro* control of food-borne and food spoilage bacteria by essential oil and ethanol extracts of *Lonicera japonica* Thunb. was reported (Rahman and Kang, 2009).

Feroniella lucida (Scheff.) Swingle is a native plant commonly found in North-eastern Thailand. Fruits and leaves are sour taste only used by local people as an ingredient for cooking soup and chili paste. The oil from plant leaves was reported to have anti-bacterial activity against *Mycobacterium tuberculosis* with the minimum inhibitory concentration (MIC) at 100 µg/mL (Supudompol, 2009). Since each *Feroniella lucida* tree found in its natural habitat, yields plenty of

fruits from our survey, the mature fruit has the average diameters of 5.73-7.00 cm and 80-115 g wet weight, and composes of peel (2.04 mm thickness and 7.22 g wet weight), hard pericarp (7.55 mm thickness and 77.82 g wet weight), and white fruit pulp with yellowish seeds (29.19 g wet weight), without any use. The mature fruit is thus attractive for essential extraction. In this study, the essential oil from the peel of mature fruits of *Feroniella lucida* (Scheff.) Swingle having fragrance suitable for developing skin care products, was investigated for its chemical composition, antibacterial activity, and mode of antibacterial action against *Staphylococcus aureus*.

Materials and Methods

Plant Materials and Extraction of Essential Oil

Mature fruits of *Feroniella lucida* (Scheff.) Swingle were collected from wild habitats of the plant having identification data and specimens deposited at the Queen Sirikit Botanic Garden Herbarium, Thailand, with the deposition QBG No. 109480, in Buriram Province, Thailand. The collected mature fruits were washed and peeled off. Essential oil was then extracted from the peel by hydro-distillation using 1 part of deionized water added to 3 parts of the wet fruit skin, and the Clevenger apparatus (LabHeat®, Basingstoke, UK) with metal-cased heating mantles for 3 h. The hydro-distillation is the standard method having advantages of the high essential oil yield achievement and environmentally friendly process compared to the solvent extraction (Chemat and Cravotto, 2012). The distillation was also proceeded below boiling water temperature that could prevent plant material from overheating (Tongnuanchan and Benjakul, 2014). The plant leaves were also collected and compared for the essential oil yield to the fruits. The essential oil was dried with sodium anhydrous and stored at 4°C until use.

Analysis of Essential Oil Chemical Composition

The chemical composition of essential oil from *Feroniella lucida* fruits was analyzed by gas chromatography-mass spectrometry (GC-MS) using a GC-7890B/MS-7000 (Agilent Technologies, Inc., CA, USA). One µL of the oil sample was injected in a system equipped with Rtx-5MS (30 m×0.25 mm×fused silica 0.25 µm) capillary column (Restek Co., Bellefonte, PA, USA); the injector split ratio of 1:20. Helium was used as carrier gas at a constant flow of 1 mL/min. The injection port was set at 250°C and the temperature cycle used was initially 50°C, ramping at 3°C/min for 3 min to a final temperature of 250°C and kept for 15 min

with the detector at 280°C. MS 320 Bruker operating parameters: transfer line temperature: 240°C; electron impact ionization at 70 eV. The components of the essential oil were identified by comparing their mass spectra with the National Institute of Standards and Technology (NIST) Mass Spectral Search Program Version 2.0f (Stein, 2008).

Test Microorganisms

The standard test microorganisms were methicillin-sensitive *Staphylococcus aureus* (MSSA) TISTR 517 and ATCC 29213, the reference strains for antibiotic susceptibility testing according to the culture collection sources. The Gram-positive bacteria were kept at the Microbial Culture Collection and Applications Research Center, Suranaree University of Technology. Tryptic soy broth (TSB) and tryptic soy agar (TSA) (Atlas, 2010) were used for cultivation of the standard test bacteria.

Antibacterial Activity Test of Essential Oil from *Feroniella lucida* Fruits

The antibacterial activity test of essential oil was performed by the disk diffusion method (CLSI, 2006). Each test was performed three times for repeating to obtain the consistent inhibition result. The test organisms were grown in tryptic soy broth (TSB) at 35°C overnight for preparing the late log phase cultures, then diluted with normal saline (0.85%) to yield approximately 10^8 CFU/mL, and inoculated on Mueller Hinton agar (Hi-Media, Hi-Media Laboratories Pvt Ltd., Mumbai, India) plate by three-dimension swab technique. Tween 80 (0.5%) was used for dissolving obtained essential oil and for negative control. The sterile blank paper disc (6 mm in diameter, Whatman®, Whatman International Ltd., Maidstone, UK) was impregnated with 10 µL containing the total 8.0 mg/disc of *Feroniella lucida* oil, and placed on the inoculated agar, then incubated at 35°C for 24 h. The inhibition zone diameter on inoculated agar was measured. Each test was carried out with the standard streptomycin disc (10 mcg, Oxoid™, CT0047B, Oxoid Ltd., Hampshire, UK) as the positive control. All tests were performed in triplicates.

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

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of essential oil against the most sensitive tested microorganism were performed by broth microdilution assay (CLSI, 2012). The MIC is defined as the minimum level of essential oil

concentration that produces a 90% inhibition of the bacterial growth, and was determined by the optical density method (Ponce *et al.*, 2003). The active overnight cultures were adjusted to yield approximately 10^5 CFU/mL in Mueller Hinton Broth. The *Feroniella lucida* oil was dissolved in Tween 80 (0.5%) for making serial dilutions from 0.1 to 16 mg/mL. Hundred microliters per well of each diluted essential oil were added with 100 µL prepared inoculum in 96 well-flat bottom microplates, and incubated at 35°C for 24 h. The standard compounds; decanol, decanal, and decyl acetate (Chanjao Longevity Co., Ltd. Bangkok, Thailand) were also performed for comparison. The bacterial growth was measured by microplate spectrophotometer (Epoch BioTek microplate spectrophotometer, BioTek®, Winooski, VT, USA) at 530 nm for detecting turbidity of the suspensions. To evaluate MBC, the broth at dilution which was not observed the bacterial growth was spread on TSA plate using the standard spread plate technique, and incubated at 35°C for 24 h. The enumeration of bacterial colony on the agar plate was then performed, and reported as CFU/mL.

Effect of the *Feroniella lucida* Essential Oil on Bacterial Viable Cell Counts

The bacterial growth during treatment with essential oil was detected using the method as mentioned by Bajpai *et al.* (2013). The active culture of the most sensitive test bacterium to the essential oil was prepared in TSB, and incubated at 35°C for 6 h to obtain approximately 10^7 CFU/mL, then used to treat with the active essential oil sample. Tween 80 (0.5%) was used as the negative control. The test culture was incubated at 35°C for 24 h. The culture samples were collected with time interval every two hours for plate counting on TSA. The results were performed as time-killing curve.

Mode of Antimicrobial Action of the Essential Oil

Release of 260 nm-Absorbing Cellular Materials

The integrity of cell membrane was monitored by the release of cytoplasmic constituents of the cell according to the methods described by Chimnoi *et al.* (2018). The selected test bacterium was incubated for 8 h in TSB for late logarithmic growth phase. The cultivated broth was then centrifuged at 10,000 rpm for 10 min at 4°C, and washed twice with 0.1 M phosphate buffered saline (PBS comprising NaCl, KCl, Na₂HPO₄·7H₂O, and KH₂PO₄ at concentration of 80.0, 2.0, 21.70, and 2.59 g/L, respectively, adjusting pH at 7.4). The cell pellet was resuspended in 0.1M PBS (pH 7.4) and

adjusted to optical density of 0.3 at 620 nm (approximately 10^7 CFU/mL). Different concentrations of the essential oil at 1×MIC and 2×MIC were added to the cell suspensions, and without the essential oil as the negative control. The suspensions were further incubated at 35°C for 0, 30, 60, 90 and 120 min, which were immediately removed at each incubation time. The cell suspension was filtered with 0.2 µm pore size syringe filter. The supernatant was detected for its absorbance at 260 nm by spectrophotometer (SmartSpec™ 3000 Spectrophotometer, Bio-Rad®, California, USA).

Leakage of Protein

The cell integrity was also examined by determining the release of proteins into supernatant using the method described by Lowry *et al.* (1951). The bacterial cell after harvesting was adjusted to optical density of 0.3 at 620 nm (approximately 10^7 CFU/mL) in 1.0% (w/v) peptone water before treating with 1×MIC and 2×MIC of *Feroniella lucida* essential oil. The treated cells were incubated at 35°C for 0, 30, 60, 90 and 120 min. At each incubation period, the mixture was filtrated through 0.2 µm pore size syringe filter. The concentration of protein released in supernatant was determined by the method as mentioned above.

Efflux of Potassium Ion

The leakage of potassium ion from bacterial cells after treating with *Feroniella lucida* essential oil was examined using the method as stated by Paul *et al.* (2011). The test bacterial cells were prepared by the method as mentioned for detecting protein leakage, and treated with the *Feroniella lucida* essential oil at concentrations of 1×MIC, 2×MIC, and MBC. Tween 80 (0.5%) was used as the negative control. The potassium ion efflux was measured by a photometric procedure using the semi-quantitative test strips for the determination of potassium (Quantofix, Macherey-Nagel GmbH and Co. KG, Düren, Germany) after 0, 30, 60, and 120 min incubation at 35°C.

Detection of Extracellular adenosine triphosphates (ATP)

To study the efficiency of *Feroniella lucida* essential oil on the strength of tested bacterial cell membrane, the extracellular ATP concentration was determined according to the method described by Paul *et al.* (2011). Harvested cells of test microorganisms were adjusted to 10^7 CFU/mL in TSB and treated with the essential oil at 1×MIC, 2×MIC, and MBC concentrations in 1 mL total volume. The mixtures were maintained at room

temperature for 30 min, then centrifuged at 3,000 rpm for 5 min. The supernatant was kept on ice to prevent ATP loss until measurement. The extracellular ATP concentration was measured using an ATP luminescent assay kit (Sigma-Aldrich Co. LLC., MO, USA) using microplate reader (Varioskan™ LUX multimode, Thermo Fisher Scientific, MA, USA).

Alteration of Bacterial Cell Morphology

Scanning electron microscope (SEM) was used to detect the morphological change of bacterial cells. The tested bacterium was cultured in TSB at 35°C for 8 h, then treated with MIC and MBC of the essential oil, and without the oil as the negative control at 35°C for 4 h. The bacterial cells were collected by centrifugation at 3,000 rpm for 5 min at 4°C and washed once with 0.1M PBS (pH 7.4), then prepared for SEM observation according to Souren *et al.* (2011). The harvested cells were fixed with 2.5% glutaraldehyde at 4°C for 3 h, and washed with 0.1M PBS (pH 7.4). The cells were then fixed with 1% osmic acid at 4°C for 1.5 h, and washed with 0.1M PBS (pH 7.4). The cell pellet was dehydrated using series of acetone concentration (30, 50, 60, 70, 80, 90, and 100%). Finally, the specimens were sputter-coated with gold under vacuum using vacuum coater (NeoCoater, JEOL USA, Inc., MA, USA), followed by the microscopic examination using scanning electron microscope (SEM, Zeiss® Gemini, Oberkochen, Germany).

Results and Discussion

Essential Oil from *Feroniella lucida* fruits and its Chemical Composition

The light yellow color and strong unique scent of essential oil was extracted from *Feroniella lucida* fruits by hydro-distillation. The average yield of the oil was 0.72% (v/w) based on the fresh weight, compared to the average yield of 0.10% from the plant leaves.

Twenty three different chemical compounds were detected in the total essential oil extracted from *Feroniella lucida* peel, which could be classified into the six groups of components; alcohol, aldehyde, ester, epoxide, saturated fatty acid, and terpene (Table 1). Ester presented at the high proportion of the oil comprising decyl acetate as a major compound in the oil of 56.20% of the total peak area, also dodecyl acetate (6.81%), octyl acetate (0.72%), and benzyl benzoate (0.12%). Aldehyde presented mainly decanal (22.49%). In general, aldehyde and ester are well known as contributors to citrus flavour that using as flavour and perfume additives (Njoroge *et al.*, 2006).

The results confirmed the characteristic which strong and unique scent found *Feroniella lucida* oil. In addition, vary chemical components were found in *Feroniella lucida* oil more than the leave oil. Supudompol (2009) reported 15 chemical components found in essential oil from *Feroniella lucida* leave, particularly β -caryophyllene (26.6%), dodecanal (18.5%), decanal (16.4%), and decyl acetate (13.3%), which exhibited a weak anti-mycobacterium. From this study, eleven compounds found in essential oil from the plant fruits were similar to compounds that were reported to be found in leaves (Supudompol, 2009) but at difference concentrations.

Table 1. Chemical composition of essential oil from *Feroniella lucida* fruits analyzed using GC-MS, and classified into six group of components

Chemical component	Retention time (min)	Peak area (%)
<i>Alcohol</i>		
1-Octanol	12.09	0.46
1-Decanol	21.85	6.82
1-Dodecanol	30.14	0.61
Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-, 4,4-dimethyl-	36.53	0.79
<i>Aldehyde</i>		
Octanal	9.18	0.03
Nonanal	13.46	0.06
Decanal	18.85	22.49
8-Hexadecenal, 14-methyl-, (Z)-	53.14	0.17
<i>Ester</i>		
Octyl acetate	18.93	0.72
Decyl acetate	28.70	56.20
Dodecyl acetate	35.71	6.81
<i>Epoxide</i>		
Caryophyllene oxide	34.30	1.84
Isoaromadendrene epoxide	37.49	0.15
<i>Saturated fatty acid</i>		
n-Hexadecanoic acid	47.30	0.11
<i>Terpene</i>		
Decane	13.25	0.31
α -Bergamotene	29.05	0.05
α -Caryophyllene	29.68	0.76
β -Farnesene	30.49	0.06
β -Bisabolene	31.40	0.28
α -Bisabolene	32.49	0.14
Longipinocarvone	33.70	0.54
Farnesol	39.10	0.10
Phytol	51.68	0.04

Antibacterial Activity of *Feroniella lucida* Oil

Essential oil from *Feroniella lucida* fruits had the potential to inhibit Gram-positive *Staphylococcus aureus* ATCC 2913 and TISTR 517 showing range of inhibitory zone from 11.22 to 25.93 mm with more than 50% of inhibition compared to the standard streptomycin disc (10 mcg) (Table 2). Essential oils have been reported to exhibit a greater effect of inhibiting the growth of *Staphylococcus aureus* (Chorianopoulos *et al.*, 2004). Normally, essential oils performed the potential inhibition to Gram-positive bacteria because the compounds could directly interact with the components of Gram-positive bacterium cell membrane (Cimanga *et al.*, 2002). However, the ability to inhibit growth of microorganisms of essential oils depending on the chemical compounds found in essential oils which those compounds could act as synergistic properties with other compounds or effect alone by main compound in highest proportion in essential oil (Bakkali *et al.*, 2008).

Table 2. Inhibition effect of *Feroniella lucida* essential oil (8 mg/disc) against two strains of *Staphylococcus aureus* using the standard streptomycin disc (10 mcg, Oxoid™) as the positive control

Test microorganism	Inhibition zone diameter (mm)	
	Streptomycin	Essential oil
<i>Staphylococcus aureus</i> ATCC 29213	12.39	11.22
<i>Staphylococcus aureus</i> TISTR 517	17.28	12.20

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

The inhibitory effect of *Feroniella lucida* oil and the efficacy to kill bacteria as MIC and MBC were investigated. Results showed that the oil was able to inhibit *Staphylococcus aureus* ATCC 29213 and TISTR 517 with higher than 50% inhibition revealing as MIC values (Table 3). Bakkali *et al.* (2008) stated that the essential oil was well known to have effect against wide range of microorganisms. *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes* were reported to be the most susceptible Gram-positive bacteria to essential oils, when compared to *Escherichia coli*

and *Salmonella* Enteritidis, the Gram-negative bacteria (Chorianopoulos *et al.*, 2004). The penetration of the oils to the bacterial cells have proposed to be main factor to relate the exhibit inhibitory activity on the functional properties of the cell, and to their lipophilic properties of the bacterial cell (Fisher and Phillips, 2006; Bajpai *et al.*, 2012). Hydrophobicity of the oils and their chemical components were the important property allowing the effect to bacterial cell by separating the lipid of bacterial cell membrane resulting in cell growth inhibition (Friedly *et al.*, 2009). Conversely, properties of hydrophilic cell wall of Gram-negatives were not conducive to the influx of hydrophobic molecules as essential oils to the cell resulting in more resistant than Gram-positive bacteria (Wiener and Horanyi, 2011).

The activities could be attributed to major components; decanol, decanal, and decyl acetate, present in the oil. Standard compounds of decanol, decanal, and decyl acetate were used for determining MIC and MBC compared to *Feroniella lucida* essential oil (Table 3). Decyl acetate showed the lowest antimicrobial activity with over 16 mg/mL to *Staphylococcus aureus* ATCC 29213, while decanal had moderate effect as MIC and MBC to the bacterium at 0.25 and 2.0 mg/mL, respectively. Decanol has the strong effect against the strain ATCC 29213 considered by MIC and MBC at 0.016 and 0.032 µg/mL, respectively (Table 3). From these results, decanol tended to be the important active compound in essential oil from *Feroniella lucida* fruits. Kubo *et al.* (1993) reported the effect of long chain alcohol decanol which had the strongest effect against *Streptococcus mutans* ATCC 25175, the Gram-positive coccus, with MIC and MBC at 6.25 and 12.25 µg/mL, respectively. Decanol also could strongly inhibit the Gram-positive bacteria; *Bacillus subtilis*, *Propionibacterium acnes*, and *Staphylococcus aureus* with less than 0.05 mg/mL (Kubo *et al.*, 1995). The essential oil from *Morinda lucida* fruits was reported to confirm the existence of alcohol and some terpene compounds; (E)-phytol (14.80%), n-octanol (6.19%) and β-caryophyllene (5.54%), showing the efficient antibacterial activity against *Staphylococcus aureus* (Prévost *et al.*, 2018).

Decanal was also reported as one of major components found in sweet orange oil, and had both bacteriostatic and bactericidal activity against Gram-positive bacterium (*Staphylococcus aureus*), Gram-negative bacterium (*Escherichia coli*) and fungi (*Saccharomyces cerevisiae* and *Aspergillus niger*) with less than 0.2 mg/mL (Liu *et al.*, 2012). The essential oil extracted from leaves of *Feroniella lucida* could inhibit *Mycobacterium tuberculosis* with MIC at 0.1 mg/mL (Supudompol, 2009).

Essential oil with terpene and phenolic compounds may contribute a strong antibacterial activity (Liu *et al.*, 2012).

The saturated fatty acid compound decanol at 50 mg/mL has been considered causing skin irritation in hairless rat (Kanikkannan and Singh, 2002). However, decanol was not the major component found in the *Feroniella lucida* fruit oil, and the MIC and MBC against *Staphylococcus aureus* ATCC 29213 and TISTR 517 were only 0.125 and 2.0, and 0.5 and 16.0 mg/mL, respectively, which was less than the concentration reported to irritate skin.

Table 3. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Feroniella lucida* essential oil against two strains of *Staphylococcus aureus* compared to standard compounds; decanol, decanal, and decyl acetate

Test microorganism	MIC/MBC			
	Essential oil (mg/mL)	Decanol (µg/mL)	Decanal (mg/mL)	Decyl acetate (mg/mL)
<i>Staphylococcus aureus</i> ATCC 29213	0.125/ 2.0	0.016/ 0.032	0.25/ 2.0	>16.0/ >16.0
<i>Staphylococcus aureus</i> TISTR 517	0.5/ 16.0	nd/nd	nd/nd	nd/nd

nd, Not determined

Time-Killing Curve of the Sensitive Test Bacterium by the Essential Oil

The essential oil was investigated for the time-kill curve of the Gram-positive bacterium *Staphylococcus aureus* ATCC 29213 (Figure 1). One and two-fold of the minimum inhibitory concentration (1×MIC and 2×MIC) and the minimum bactericidal concentration (MBC) of the essential oil were applied. The treatment using MBC (2.0 mg/mL) indicated the rapid decrease in numbers of the strain ATCC 29213 cells within 2 h, and none of cells was detected after 2 h. While at 1×MIC (0.125 mg/mL) and 2×MIC (0.25 mg/mL) the results showed slight decrease in cell numbers in 2 h. The oil concentration at 2×MIC provided the higher efficiency to inhibit the bacterial growth than 1×MIC. These results confirmed that the *Feroniella lucida* essential oil had significant effect on the growth of *Staphylococcus aureus* ATCC 29213. After 6 h incubation, the bacterial growth was slightly increased, and stable at around 10⁷ CFU/mL until 24 h for 1×MIC but the growth was stable at around 10³ CFU/mL after 6 to 24 h for 2×MIC. The time-killing curve of the test

bacterium using 1×MIC and 2×MIC exhibited less effect after 6 h indicated that the oil did not completely inhibit the bacterial cells, but increased the lag phase time of the growth curve. This result correlated to the study of fennel oil to inhibit *Shigella dysenteriae* CMCC 51252 (Diao *et al.*, 2014).

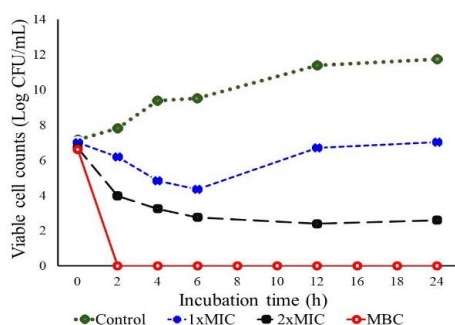


Figure 1. Viable cell counts of *Staphylococcus aureus* ATCC 29213 after treating with *Feroniella lucida* essential oil at 1×MIC (0.125 mg/mL), 2×MIC (0.25 mg/mL), and MBC (2.0 mg/mL) for 24 h

Mode of the Essential Oil on Antimicrobial Action

The effect of essential oil on *Staphylococcus aureus* ATCC 29213 cell membrane was investigated by detecting the release of 260 nm-absorbing cellular materials (DNA or RNA), the leakage of protein, the efflux of potassium ion, and extracellular ATP concentration out of the bacterial cells. The morphological alteration in cell wall was also considered.

Release of 260 nm-Absorbing Cellular Materials

No leakage of 260-absorbing cellular materials (nucleic acid) was detected from the exposure of *Feroniella lucida* essential oil at 1×MIC (0.125 mg/mL), 2×MIC (0.25 mg/mL), and MBC (2.0 mg/mL) for 30, 60, 90, and 120 min to *Staphylococcus aureus* ATCC 29213 cells comparing to the control without the essential oil. The essential oil was not affected the integrity of the Gram-positive *Staphylococcus aureus* ATCC 29213 cell. The measurement of 260 nm-absorbing materials leakage demonstrated cell lysis by expected leakage of nucleic acids, DNA, and/or RNA (Huang *et al.*, 2018).

Leakage of Protein

The release of cellular protein from *Staphylococcus aureus* ATCC 29213 cell after treatment with *Feroniella lucida* essential oil at

1×MIC (0.125 mg/mL), 2×MIC (0.25 mg/mL), and MBC (2.0 mg/mL), was detected (Figure 2). The cellular protein leakage increased when increasing the concentration of the oil in 30 min comparing to control (without the essential oil). The protein (75.63 µg/mL) was detected in 30 min of 1×MIC treatment, and the amount of protein slowly increased to the highest of 174.05 µg/mL in 120 min. While at 2×MIC, the protein concentration was slightly higher than 1×MIC, which was 107.91 µg/mL in 30 min and 268.04 µg/mL at the end of treatment time, 120 min. The MBC also exhibited the strong effect of the oil to *Staphylococcus aureus* ATCC 29213 cell with the cellular protein leakage of 206.32 and µg/mL in 30 min, 310.13 and 240.51 µg/mL at 60 and 120 min. The result of protein leakage represented the possibility of the essential oil composition to act on proteins embedded on bacterial cell membrane. The essential oil might bind to the cell membrane resulting in disturbance of enzymes such as ATPases.

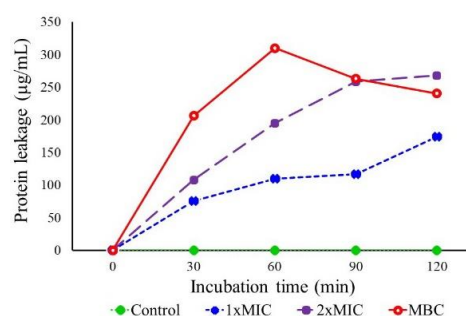


Figure 2. Leakage of cellular protein from *Staphylococcus aureus* ATCC 29213 cells after treating with essential oil from *Feroniella lucida* fruits at 1×MIC, 2×MIC, and MBC for 24 h

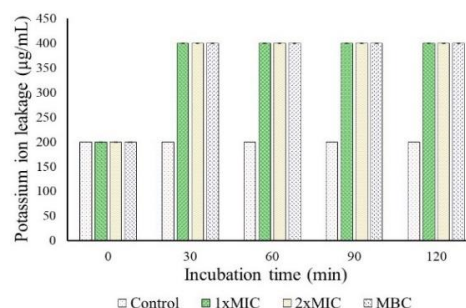


Figure 3. Leakage of potassium ion from *Staphylococcus aureus* ATCC 29213 cells after treating for 24 h with essential oil from *Feroniella lucida* fruits at 1×MIC, 2×MIC, and MBC

Efflux of Potassium Ion

The leakage of potassium ion (400 µg/mL) from *Staphylococcus aureus* ATCC 29213 cells occurred after treating with *Feroniella lucida* essential oil for 30 min (Figure 3). The potassium ion efflux was consistent during incubation for 120 min (Figure 3).

Extracellular ATP Concentration

The effect of essential oil from *Feroniella lucida* fruits on the leakage of extracellular ATP from in *Staphylococcus aureus* ATCC 29213 cells were detected. When treated the bacterial cells with the essential oil at 1×MIC, 2×MIC, and MBC for 24 h, the extracellular ATP concentrations of 2.22, 1.68, and 1.10 ng/mL were accumulated. None of extracellular ATP was detected for the untreated cells. Paul *et al.* (2011) also reported the anti-bacterial potential of the essential oil and extracts of *Trachyspermum ammi* fruits against food borne and spoilage bacteria including *Staphylococcus aureus* ATCC 6538 as one of the test bacteria but the most sensitive bacterium to the oil was *Bacillus subtilis* ATCC 6633. The effect of *Trachyspermum ammi* oil on extracellular ATP concentration, release of cell constituents, and potassium ions from the bacterial cell, were only performed with *Bacillus subtilis* ATCC 6633 (Paul *et al.*, 2011)

Based on the results of measurement, the leakage of various substances from *Staphylococcus aureus* ATCC 29213 cells after treated with *Feroniella lucida* essential oil were found. Protein, potassium ion, and extracellular ATP were detected when treated the strain ATCC 29213 with 1×MIC, 2×MIC and MBC. No leakage of 260-absorbing cellular materials (nucleic acid) was detected. The essential oil from *Feroniella lucida* could disturb the bacterial cell wall resulting in unable to grow regularly. However, effects of essential oil were well known including destabilization of the phospholipid bilayer, the destruction of the plasma membrane function and composition, the loss of vital intracellular components and the inactivation of enzymatic mechanisms (Nazzaro *et al.*, 2013).

Effect of Essential Oil from *Feroniella lucida* Fruits on Bacterial Cell Morphology

The SEM analysis was used to investigate physical and morphological alterations in cell wall of the sensitive test bacterium, *Staphylococcus aureus* ATCC 29213 (Figure 4). Essential oil of *Feroniella lucida* fruits caused morphological alterations on the cell wall of the bacterium. Cells of the bacteria were changed with more crease and furrow at concentration of essential at MIC while

greater concentration as MBC. Sikkema *et al.* (1995) described effect of the essential oil on the alteration of membrane lipid composition, it was thought to be a compensatory mechanism to counter the lipid disordering effects of the treatment agent.

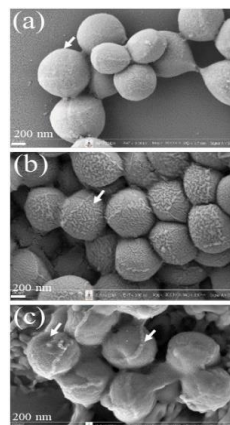


Figure 4. SEM micrographs of *Staphylococcus aureus* ATCC 29213 cells (arrows) without treating with *Feroniella lucida* essential oil (A), and treating for 4 h with the essential oil at 1×MIC (B) and MBC (C), 30,000× magnification

Conclusions

The yield of hydro-distilled essential oil from the peel of *Feroniella lucida* (Scheff.) Swingle was approximately 0.72% (v/w) based on the fresh weight, which was much more amount than the extract from the plant leaves having the average yield of 0.10% (v/w). The major components of the essential oil from the peel were found to be decyl acetate, ethyl decanoate and decanal, which associated strong unique fragrance. The oil had ability to inhibit two strains of Gram-positive bacterium, methicillin-sensitive *Staphylococcus aureus* (MSSA) TISTR 517 and ATCC 29213, particularly disturbing the strength of cell wall and cell membrane of the ATCC 29213. The essential oil had a significant effect to inhibit the bacterium growth within 2 h, and caused protein, potassium ion, and adenosine triphosphates (ATP) leakages, but it had no effect on the leakage of DNA and RNA. These results support the use of *Feroniella lucida* fruits more than leaves for essential oil extraction thus the plant growth would not be disturbed from the loss of leaves. The application consideration of the oil for both cosmetic and food sectors. The essential oil containing high proportion of ester (decyl acetate) and aldehyde (Decanal)

might be suitable to apply in some cosmetic and perfume products, which need to be further investigated.

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