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STUDY OF ANTIBACTERIAL PROPERTIES OF JAPANESE MINT (MENTHA ARVENSIS L.) ESSENTIAL OIL ON THE BEGINNING OF SHOWER GEL PRODUCTION

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Abstract

This study aims to exploit the antimicrobial potential of Japanese mint oil (*Mentha arvensis* L.) to apply for the beginning of shower gel production. The strain used was *Staphylococcus aureus* that commonly causes skin infections in humans. Shower gel formulation used Sodium Laureth Sulfate (SLES) and Cocamidopropyl betaine (CAPB) as surfactants, the concentration of Japanese mint oil in shower gel formulation was identified through the determination of Minimal Inhibitory Concentration (MIC), Minimal Bactericidal Concentration (MBC) and Time-kill assay. Antibacterial activity of Japanese mint shower gel was determined by Agar well diffusion method. The results indicated that Japanese mint oil inhibited *S.areus* at 1.25 mg/ml (MIC), the MBC value was 10mg/ml that killed 99.9% of *S.aureus* in less than 30 minutes of interaction. Shower gel formulation contained 1%w/v Japanese mint oil has the diameter of inhibition zone was 35 mm. This study showed that shower gel contained Japanese mint essential oils had antibacterial activities superior to conventional shower gel.

Keywords: antimicrobial, CAPB, mint oil, shower gel, SLES, *Staphylococcus aureus*.

1. INTRODUCTION

Skin is the habitat of many opportunistic microorganisms such as *Staphylococcus spp., Streptococcus spp., Propionibacterium spp.,...* The chemical changes in the skin lead to the breaking of the immune barrier, facilitate microorganisms to spread through and causes diseases (Tognetti et al, 2012). In particular, *Staphylococcus aureus* is the common germ causes skin infection such as Impetigo, Folliculitis, Furuncles, Carbuncles, Erysipelas and Cellulitis (Ni Riain,2008).

Antimicrobial chemicals have long since been applied in the personal care products to prevent pathogenic bacteria. But notwithstanding this, in November 2016, The Food and Drug Administration (FDA) has banned soaps and personal care products that containing Triclosan and 18 other chemicals with the reason were these chemicals not showed markedly effective than ordinary soap and posed to the spread of antibiotic resistance throughout the environment (McNamara et al, 2016), (Yee et al, 2016). Thus, examining the potential exploitation of natural antimicrobial compounds is extremely necessary.

Before the role of microorganisms in disease pathogenesis was understood, the treatment of diseases commonly used plant-based medicines essential oils had antibacterial properties. The plant therapy tended to decrease when the antibacterial sulfur drugs were detected with selective bactericidal activity and being susceptible to bacteria. Yet, on the antibiotic resistance situation was becoming more and more popular, Using plant-based medicines gradually revived with mong muốn the desire for safety antibacterial compounds (Thormar H, 2011). Japanese mint (*Mentha arvensis* L) oil was one of the plant-based medicines used for a long time with menthol was the main ingredient that likely destroyed approximately 100% plasmids (Schelz et al, 2006). According to Zhang and Coutinho, the extract of *Mentha arvensis* L. against bacteria antibiotic resistance by causing protein leakage, damage the membrane and promote the oxidation reaction (Coutinho et al, 2009) (Zhang et al, 2015). The objective of this study is to find out the bactericidal concentrations of Japanese mint essential oil for *Staphylococcus aureus* as the basis for the beginning of shower gel production

2. MATERIALS AND METHODS

2.1 MATERIALS

Plant Material. Japanese mint essential oil was extracted from fresh Japanese mint (*Mentha arvensis* L.) leaves were provided by Chu Ong Vang Co.(Ho Chi Minh City, Viet Nam). Fresh mint leaves were dried at 50^0C. A total of 8g dried leaves were distilled with DAB-type apparatus consisting of a 1L distillation bottle and a 3 mL graduated receiver. 250 mL of distilled water was used. The distillation time was 45 minutes.

Bacteria. The bacteria strain used in this study was *Staphylococcus aureus* ATCC 12600 provide by Binh Phuoc Hospital (Binh Phuoc, Viet Nam). Mueller Hinton Broth (MHB) medium was provided by Himedia (India).

2.2 METHODS

Determination Japanese mint oil composition by Gas Chromatography/Mass Spectrometry. The Japanese mint oil was sent to Institute of Chemical Technology (Ho Chi Minh City, Viet Nam) to analyze the composition by Gas Chromatography/Mass Spectrometry- (GC/MS). Using DB 5-MS (30m x 0.25mm -0.25µm) with helium as carrier gas. GC oven temperature was held at 70^oC for 5 min, then programmed to 250^oC at a rate of 10^oC/min and held for 3 min.The reference points in the calculation of relative retention indices (RRI) was Hexanes. Split ratio was adjusted at 20:1, the injector temperature was 250^oC (split injection; 1 µL). Mass range was formed m/z 10 to 550. Using data from National Institute of Standards and Technology (NIST) to identify individual components.

Determination of Minimal Inhibitory concentration (MIC) and Minimal Bactericidal Concentration (MBC). (Lertsatitthanakorn et al, 2014) Using Broth microdilution method to determine MIC and MBC value of Japanese essential oil on resistance to *S.areus.* Mueller Hinton Broth (MHB) with 10% Dimethyl sulfoxide (DMSO) was used to dissolve essential oil. The essential oil concentrations were prepared form 20mg.mL^{-1} to 0.0078mg.mL^{-1}. 90 microliter of each oil sample were prepared in 96-well plate. Then adding 10 microliters of *S.areus* culture to each well that the final concentration was approximate 10^7CFU/ml. The test used *S.areus* in MHB

and MHB alone as positive and negative growth control. Incubating plates at 37^0C for 24 hours to determine the MIC value. The MIC was defined as the lowest essential oil concentration that inhibited visible growth of bacteria.

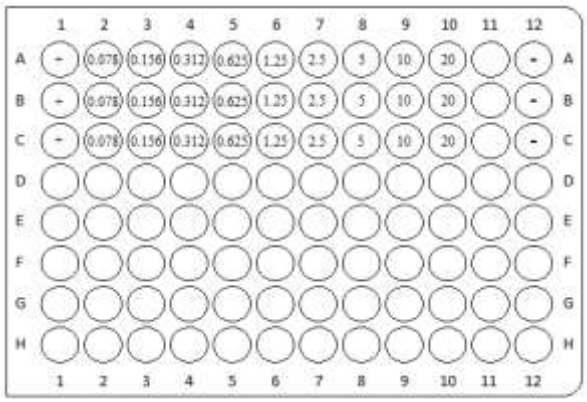

Figure 1. *Diagram arrangement of essential oil concentration in 96-well plate*

MBC value was determined by spotting 100 microliters of broth from each well onto Mueller Hilton Broth-agar. Incubating Petri plates at 37^0C for 24 hours. The MBC was defined as the lowest concentration sterilized at least 99.9% of the initial inoculum.

Time-kill assay. According to the method of Lertsatitthanakorn et al. Assessing the rate of bactericidal activity at MBC in one hour and determining the time required by essential oil to kill the *S.aureus*. The experiment was performed in triplicate. Results are presented graphically as time-log survivors curves with bars resenting the standard deviation (Time-log survivor curve).

Shower gel formulation. (Ananthapadmanabhan et al,2004); (Lertsatitthanakorn et al, 2014) In addition to antibacterial activities, Japanese essential oil also used as a fragrance. Shower gel formulation used SLES as main surfactant and CAPB as co-surfactant with the ratio of 2:1, Rheology modifier was Sodium chloride (NaCl), emollient was glycerin. The pH was adjusted with citric acid.

Table 1. *Shower gel formulation*

Ingredients	Mass
Sodium Laureth Sulfate (SLES)	36.75g
Cocamidopropyl betaine (CAPB)	18.25g
Sodium chloride (NaCl)	5.5g
Citric acid	0.05g
Glycerin	18g
Japanese mint essential oil	MBC value

Deionized water	Adjusted to 250ml

The physical stability of shower gel was performed by heating cooling cycling method. One cycle included keeping the shower gel at 4^{o}C for 24 hours and 45^{o}C for 24 hours. Recording color, odor, pH, viscosity and physical stability after freshly prepared and after five cycles of heating-cooling cycling.

Determination of shower gel antibacterial activities. Antibacterial activity of Japanese mint shower gel was determined by Agar well diffusion method. Spreading S.aureus inoculum (approximate 10^{8}cfu/ml) onto MHB-agar by Swab disinfection . Using Pipetman Tips to create 8mm diameter of agar well. 100 microliter of shower gel was prepared in well. The test used 95% ethanol and shower gel without essential oil as controls. Petri plates were incubated at 37^{o}C for 24 hours. Shower gel antibacterial activities were determined by the diameter of growth inhibition zone.

3. RESULTS AND DISCUSSION

Determination Japanese mint oil composition by Gas Chromatography/Mass Spectrometry. The oil content obtained from hydrodistillation was 5.42%v/w (by dry weight), with clear, yellow and characteristic menthol mint. As a result, GC/MS was identified 13 compounds (Table 2) with oxygenated monoterpenes were L-Menthol (71.94%) and Menthone (15.52%) as the main constituents. The rest were hydrocarbon monoterpene, hydrocarbon sesquiterpenes, and oxygenated compounds.

Table 2. *Japanese mint (Mentha arvensis L) essential oil composition determined by GC/MS*

No.	RT* (Min)	Composition	Content (%)	Type**
1	5.55	1R-α – Pinene	0.85	MH
2	6.76	1S-α – Pinene	1.77	MH
3	6.98	1-Naphthalenecarbonitrile, 2-methoxy-	0.82	OC
4	7.18	2-Dibenzofuranamine	0.60	OC
5	8.07	D-Limonene	1.51	MH
6	10.97	L-Menthone	13.53	MO
7	11.14	D-Menthone	1.99	MO
8	11.22	L-Menthol	71.94	MO
9	12.51	Pulegone	4.36	MO
10	12.78	3-Cyclohexen-1-one,2-isopropyl-5-methyl-	1.07	OC
11	13.57	2,4-Dimethyl-6-phenylpyridine	0.61	OC
12	15.48	3,5-Dimethyl-4-phenylpyridine	0.61	OC
13	16.35	Germacrene D	1.19	SH

*RT: Retention Indices.

**MH: Hydrocarbon Monoterpene; MO: Oxygenated Monoterpene; OC: Oxygenated Compound; SH: Sesquiterpene Hydrocarbon.

According to Ulubelen et al, hydrocarbon monoterpene and oxygenated monoterpenes damage the cell organelle through the inhibition of ion transport and respiration (Ulubelen et al, 1994). In addition, the oxygenated monoterpenes display higher antibacterial activities than hydrocarbon monoterpenes (Knobloch et al, 1986). Lambert et al described that antibacterial activities of essential oil in gram-positive was highly effective (Lambert et al, 2001). Specifically, *Mentha arvensis L.* essential oil was displayed higher antimicrobial activity in gram-positive (*Staphylococcus aureus, Streptococcus pyogenes)* gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) (Zhang et al, 2015). Whereby, *S.aureus và S.pyogenes* were the strains commonly cause infections on human skin (Ni Riain, 2008). This highlights the potential of *Mentha arvensis* L. essential oil in the application of antibacterial products for skin.

Determination of Minimal Inhibitory concentration (MIC) and Minimal Bactericidal Concentration (MBC). MIC value was determined by the visible growth of bacteria that displayed by the opacity of the suspension or the formatting of button growth (Coyle, 2005). From the concentration at 1.25mg/ml (approximate 1.38µl/ml) in 6th well onwards, there was no formation of button growth. Hence, 1.25mg/ml was the MIC value. When Spreading suspension of the 6th well onwards onto MHB-agar, the 9th (10mg/ml) was showed the capable of killing 99.9% of the initial inoculum (Figure 3). Thus, the MBC value was 10mg/ml.

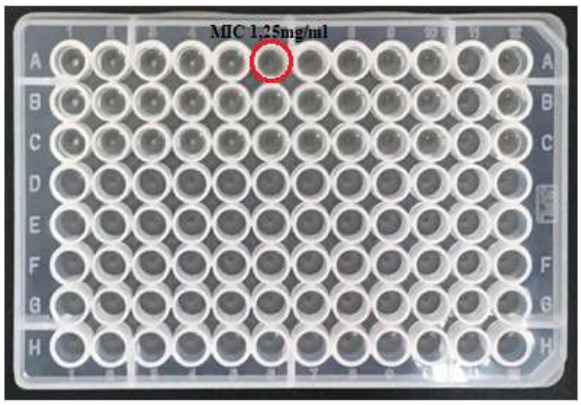

Figure 2. *Minimal Inhibitory Concentration (MIC)*

Figure 3. *Minimal Bactericidal Concentration-MBC*

This MIC was lower about 9 times than the MIC value of *Mentha arvensis L.* essential oil determined by agar diffusion method (12,5µl/ml) (Bokhari et al, 2016). This difference is due to the total amount of the oxygenated monoterpenes of essential oil in this study was 91.82%, higher than the essential oil was used in the study of Bokhari (87.42%). Accordingly, the oil that had the total content of oxygenated monoterpenes higher would demonstrate stronger antibacterial activities. In addition, Japanese mint essential oil in this study also displayed the antibacterial activities with *S.aureus* stronger than peppermint (*Mentha piperta* L.) essential oil (MIC:1.6 mg/ml) (Inouye et al, 2001) and Spearmint (*Mentha spicata L.*) essential oil (MIC: 2.25mg/ml) (Hammer et al, 1999).

Time-kill assay. The result of the time-kill test was described by log survivor of *S.aureus*. The 99.9% reduction was reported as 3 log reduction. (Kuo Yann Lai, 2006). According to Figure 4, Japanese mint oil concentration at MBC value was likely to kill nearly 99.9% (3 log) in 20 minutes and higher than 99.9% within 30 minutes. This result showed that Japanese mint oil resistant *S.aureus* stronger than Cinnamon essential oil (*Cinnamomum zeylamicum*) (Lertsatitthanakorn et al, 2014), Tea tree oil (*Melaleuca alternifolia)* (May et al, 2000).

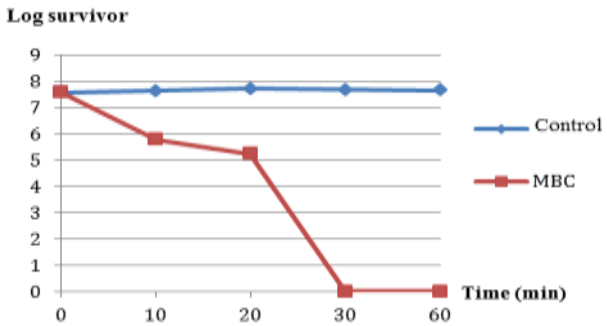

Figure 4. *The time-killed curve of Japanese mint (Mentha arvensis L.) oil on S.aureus*

Shower gel formulation. Japanese mint essential oil was added to the shower gel at 10 mg/ml (MBC) equivalent to 1% v/v. This totally consistent with the limit

concentration of mint essential in the rinse-off products (Nair,2001). Shower gel formulation was according to Lertsatitthanakorn with Sodium Lauryl Sulfate (SLS) was replaced with SLES and CAPB. SLS was the anion surfactant that containing alkyl sulfate, these alkyl sulfates tended to link with the protein keratin on the skin caused skin irritation (Ananthapadmanabhan et al,2004). There have been many studies showed that SLS cause irritation when applied to the skin at 10-14% (Loden et al, 1997);(Patterson et al, 1999). Instead, SLES was anion surfactant containing alkyl sulfate ethoxylated- Less linked with keratin than alkyl sulfate radicals. Thus, SLES was milder with skin than SLS. In addition, the combining of SLES and CAPB at rate 2:1 also reduced skin irritation skin irritation of SLES (Ananthapadmanabhan et al,2004). The change of surfactant lowered the pH, therefore need to reduce the amount of citric acid in order to achieve the initial pH.

Table 3. *Physical stability of shower gel after the 5th heating-cooling cycle*

Physical properties	Freshly prepared	After the 5th heating-cooling cycle
Color	Clear	Clear
Odor	Characteristic menthol mint	Characteristic menthol mint
pH[a]	5.32±0.005	5.27±0.015
Viscosity[a]	6,763±40 cP	5243±20 cP
Physical stability	Stable	Stable

[a] The experiment was performed in triplicate (Mean±SD, n=3)

Shower gel with pH at 5.3 was closed to the skin's pH balance (5.5) therefore less likely to cause skin irritation. The viscosity of shower gel was 6.763 cP meet the standards of the viscosity of shower gel (5,000cP -15,000cP) (Lai, 2005). Shower gel formulation was stable on by remaining the same color, odor with a slightly changes in pH and viscosity after heating cooling storage.

Determination of shower gel antibacterial activities. Shower gel contained essential oil at 10mg/ml (MBC) was spread onto MHB-agar to determine antibacterial activity compared with conventional shower gel and 95% ethanol. As a result, adding Japanese essential oil to shower created the superior antibacterial activities with the diameter of inhibition zone was 35mm.

Table 4. *Diameter of inhibition zone of Japanese mint essential oil*

Well	Diameter of inhibition zone **(mm)***
95% ethanol[a]	4.1±0.2
Conventional shower gel[a]	13.6±1.2
Japanese mint shower oil gel[a]	35±2.6

* Diameter of inhibition zone= Diameter of large zone-Diameter of zone

[a] The experiment was performed in triplicate (Mean±SD,n=3)

Figure 5. *Diameter of inhibition zone of Japanese mint oil shower gel*

Shower gel broader inhibition zone than ethanol due to the antibacterial activities of SLES và CAPB. According to Kabara, Low concentrations of surfactants was used to provide energy for the growth of bacteria but at concentrations higher than 10%, surfactants had the ability to kill Gram-positive such as *Staphylococci*, *E.coli* and some seminarians spores (Kabara, 1997). The inhibition zone of Japanese mint oil shower gel (35mm) was markedly wider than the conventional shower gel (13.6mm) and broader than the antibacterial shower gel of Cinnamon oil (20.7mm) 1,69 times. (Lertsatitthanakorn et al, 2014). This suggests that using Japanese mint essential oil as antibacterial agent in shower gel was perfectly feasible. additionally, Japanese mint oil shower gel also displed the higher antibacterial activities than antibacterial soap such as Detol (10mm), Life Buoy (13mm), Safe Guard (20mm) (OBI, 2014).

4. CONCLUSION

The *Mentha arvensis* L. essential oil had strong resistant to gram-positive that commonly caused skin infection such as *Staphylococcus aureus* dependent on two type of monoterpene, hydrocarbon monoterpene and oxygenated monoterpene. In particular, The higher total content of oxygenated monoterpenes was, the stronger of antibacterial activities was. The Japanese oil concentration at 10mg/ml was likely killing 99.9% of *S.aureus* within 30 minutes. Shower gel contained 1%v/w (10mg/ml) of Japanese mint oil displayed superior antibacterial activities than Cinnamon shower gel *Cinnamomum zeylamicum* and antibacterial soap such as Detol, Life Buoy và Safeguard.

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