Efficacy of essential oil formulations against malodor causing bacteria

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Abstract

Malodor is an unpleasant sense induced by overgrowth of malodor causing bacteria. These bacteria can produce volatile organic compounds from their normal metabolisms. To reduce malodor, good hygiene combined with antimicrobial agents are suggested resulting in lowering amount of foot odor-producing bacteria. Essential oils have many favorable properties including unique senses and antimicrobial activities. In this study, eight essential oils were tested against five malodor causing bacteria. From the results, lemongrass oil exhibited the lowest MICs and MBCs. Meanwhile, clove oil and cinnamon leaf oil showed lower potency than lemongrass oil. Therefore, lemongrass oil was selected to be developed self-emulsifying formulations for foot bath. Four surfactants were chosen to test the compatibility with lemongrass oil. Only Tween 20 and Span 20 gave clear appearance and no phase separation after mixed with the oil and they were combined to be used in the formulation. The highest concentration of lemongrass oil in the formulation was 40% w/w. Tween 20 and Span 20 were mixed at various ratios ranged from 50:10% to 10:50% w/w. After mixing with water, all formulations could be simply emulsified with the particle size of less than 200 nm and no phase separation was observed. The formulation containing 40:50:10% w/w of oil:Tween 20:Span 20 demonstrated the most potent antibacterial activity and its MICs and MBCs were lower than lemongrass oil alone due to the formation of very fine emulsion. This formulation showed promising potential for the development of foot bath self-emulsifying emulsion formulation to be used in Thai spa.

Keyword: Essential oil, antibacterial activity, self-emulsifying, malodor causing bacteria, foot bath

1. INTRODUCTION

The layers of skin are keeping moist in the body and protecting body from harmful environments including pathogens. Skin contains two types of sweat glands which are eccrine and apocrine glands. Eccrine glands are found all over the body especially on the palms and feet while apocrine glands can be found at specific areas such as armpits. Skin is also the living place for normal flora bacteria. These bacteria in beneficial surrounding by secretions from sweat glands create body odor. In particular to feet and armpits where high density of microorganisms are living, the bacteria produce unpleasant odor frequently resulting in uncomfortable sense of human. Malodor can be caused by bacteria overgrowth in optimum environment[1]. To reduce odor, some strategies such as, good hygiene combined with deodorant, antiperspirant or even antimicrobial agents, were suggested to decrease amount of bacteria[1]. Some species of bacteria were reported to release specific characteristic smell *in vitro* caused by their normal metabolism.

Volatile organic compounds (VOCs), including fatty acids and their derivatives, such as isovaleric acid and isobutyric acid were the primary components of foot odor[2]. Those fatty acids were produced by many microorganisms such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus* spp.[3]. Normal bacteria which can be found on

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human skin are *S. aureus* and Coryneform bacteria such as, *Corynebacterium* spp., *Brevibacterium* spp., and *Propionibacterium acnes*[4, 5]. Rennie PJ, et al., reported *Corynebacterium* spp. and *Brevibacterium* spp. involving body malodor while *Micrococcus* spp. and Propionibacteria involved foot odor production[6]. Marshall J, et al., mentioned that Micrococci, Staphylococci and aerobic Coryneform bacteria with the ability to produce exoenzymes such as lipase and proteinase were associated with foot odor[7]. Among these skin normal flora bacteria, *Staphylococcus* spp. have been well studied[8]. However, there were only a few studies of essential oils against *Micrococcus* spp. and *Brevibacterium* spp..

Essential oils are volatile substances and can be obtained from various parts of plants. Essential oils have many favorable properties such as their unique aroma, anti-inflammatory, antioxidant and antimicrobial activities, which make them popular in many industries including pharmaceutical, food, fragrance, and dermatology[8, 9]. Hydrophobicity of essential oils plays an important role in antimicrobial activity. They enable to partition to the lipid parts of bacterial cell membrane leading to the death of microbes[10]. Antimicrobial activity of essential oils varies depended on types of assays and microorganisms.

Hydrophobic property of essential oils makes them incompatible with water. To prepare a formulation containing essential oils, surfactants are required. Although many surfactants can solubilize the essential oils, however they reportedly affect the activity of essential oils[11, 12]. Tween 80 was often used as an emulsifier in emulsion-based formulations of essential oils. Ma Q, et al., reported the decrease of antimicrobial effect of eugenol when mixed with Tween 80[13]. Different surfactants can also exhibit different antimicrobial activity of formulations. Hammer KA, et al., demonstrated different surfactants affected inhibitory activity of tea tree oil such as, Tween 80 increased MICs of tea tree oil more than Tween 20[14]. In addition, increasing of the concentration of surfactants including Tween 80 in the formulations also decreased antimicrobial activity[13, 15]. Therefore, the objectives of this study were to test the antibacterial activity of essential oils and to determine proper surfactants which could maintain antibacterial activity of essential oils.

2. MATERIALS AND METHODS

2.1. Materials

All plant essential oils were purchased from Thai-China Flavours and Fragrances Industry Co., Ltd, Thailand. The oils employed in this study were lemongrass oil (*Cymbopogon citratus* (DC.) Stapf, part used: leaves), galanga oil (*Alpinia galanga* L., part used: rhizomes), kaffir lime oil (*Citrus hystrix* L., part used: leaves), holy basil oil (*Ocimum tenuiflorum* L., part used: leaves), sweet basil oil (*Ocimum basilicum* L., part used: leaves), cinnamon oil (*Cinnamomum zeylanicum*, part used: leaves), clove oil (*Syzygium aromaticum* L., part used: buds) and turmeric oil (*Curcuma longa* L., part used: rhizomes).

2.2. Bacterial culture collection

Microorganisms used in this study were chosen based on skin normal flora. *Bacillus subtilis* ATCC6633, *Micrococcus luteus* ATCC9341, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus aureus* ATCC25923, and *Brevobacterium* spp. were used in this study. *Brevibacterium* spp. was purchased from the culture collection of the Thailand Institute of Scientific and Technological Research (TISTR). Optimum culture temperature of all tested microorganisms was 37°C except for *Brevibacterium* spp. which required at 30°C. All bacteria were cultured in tryptic soy broth. For the determination of minimum inhibitory concentrations (MICs), Mueller Hinton broth was used as a culture medium.

2.3. Antimicrobial activity assay of essential oils

The stock solution of the essential oils were prepared at 1% v/v by dissolved in DMSO and then diluted with water. DMSO was used at the limit of not more than 5%v/v of total volume. The MICs were determined by broth microdilution assay. Briefly, single isolated colony was selected and cultured

overnight. Bacterial cultures were adjusted to 0.5 McFarland and diluted to 10^6 CFU/ml. The stock solution of essential oils was diluted by two-fold dilution with Mueller Hinton broth and then the diluted bacterial culture was added. After 16-18 h of incubation, micro wells with clear solution were further determined for minimum bactericidal concentrations (MBCs) by streaking on Mueller Hinton agar. The agar plates were incubated overnight. The lowest concentration with no visible growth of bacteria was recorded as MBC.

2.4. Determination of compatibility of surfactants and essential oils

To formulate the small size emulsion of essential oils, the compatibility of surfactants and essential oils was determined. Span 20, PEG 400, Solutol HS15 and Tween 20 were chosen since they were often used in micro- and nanoemulsion formulations. Lemongrass oil was mixed with each surfactant at 1:9, 5:5 and 9:1 mass ratios. The appearance of the mixture was observed with naked eyes. The surfactant that gave the clear solution of mixtures was further utilized for the formulation.

2.5. Preparation of self-emulsifying emulsion formulation

A pair of surfactants which gave the clear solution was used to prepare self-emulsifying emulsion formulation. Lemongrass oil was employed in the formulation at 40% w/w while the rest component was the mixture of Tween 20 and Span 20 at different ratios varied from 50:10 to 10:50% w/w. The obtained formulations were then tested upon dilution with water at 1:160 volume ratio. The appearance of formulations was observed before and after dilution. In addition, phase separation and particle size of formulations after dilution were examined. The particle size of stable formulations was measured by Zetasizer NanoZS (Malvern Instrument, Malvern, UK) at an angle of 173°, 25°C.

2.6. Antimicrobial activity assay of formulation

The stable formulations with maximum ratio of each surfactant were selected to test for their antimicrobial activity by broth microdilution assay. The formulations were diluted with water at 1:160 volume ratio yielding the final concentration of lemongrass oil of 0.25% v/v. After that, the diluted formulations were employed in antimicrobial assay to determine MICs and MBCs as previously described. MICs and MBCs were calculated and reported as amount of essential oil in % v/v.

2.7. Statistical analysis

All experiments were performed in triplicate. Descriptive statistics was performed in this study using Microsoft Excel 2010. All results are given as mean ± standard deviation.

3. RESULTS AND DISCUSSIONS

3.1. Antimicrobial activity assay of essential oils

MICs and MBCs of tested essential oils against malodor causing bacteria were shown in Tables 1 and 2. Lemongrass oil demonstrated the lowest MICs against all tested microorganisms. MICs of lemongrass oil ranged from 0.0312% v/v to 0.125% v/v. The MICs of clove oil and cinnamon leaf oil were ranked as the second and third lowest MICs values against all tested bacteria and ranged from 0.125% v/v to 0.25% v/v and 0.125% v/v to 0.5% v/v, respectively. Sweet basil oil exhibited inhibitory effect against *B. subtilis* and *Brevibacterium* spp. at 0.5% v/v whereas turmeric oil and kaffir lime oil showed the effect against only *B. subtilis* and *S. epidermidis*, respectively, at the same concentration. However, galangal oil and holy basil oil at the maximum tested concentration did not demonstrate inhibitory activity against all tested bacteria. All essential oils exhibited antimicrobial activity were further studied for MBCs. The MBCs of lemongrass oil were found to be in the range of 0.0625-0.25% v/v depended on the species of bacteria (Table 2). Nevertheless, this oil did not exhibit bactericidal activity against *S. epidermidis* at the maximum tested concentration of 0.5% v/v. Clove oil and cinnamon leaf oil demonstrated bactericidal activity against *B. subtilis* at 0.125% v/v and

0.5% v/v, respectively. Meanwhile MBCs of these oils against other microorganisms were higher than 0.5% v/v. Sweet basil oil showed MBC against *Brevibacterium* spp. at 0.5% v/v while turmeric oil and kaffir lime oil showed higher than 0.5% v/v against the tested bacteria.

Table 1. Minimum inhibitory concentration (%v/v) of essential oils against tested microorganisms

	Minimum inhibitory concentration (%v/v)				
	B. subtilis	*Brevibacterium* spp.	*M. luteus*	*S. epidermidis*	*S. aureus*
Lemongrass oil	0.0312%	0.125%	0.0625%	0.0625%	0.125%
Clove oil	0.125%	0.125%	0.25%	0.25%	0.25%
Cinnamon leaf oil	0.5%	0.125%	0.5%	0.5%	0.5%
Sweet basil oil	0.5%	0.5%	>0.5%	>0.5%	>0.5%
Tumeric oil	0.5%	>0.5%	>0.5%	>0.5%	>0.5%
Kaffir lime oil	>0.5%	>0.5%	>0.5%	0.5%	>0.5%
Galanga oil	>0.5%	>0.5%	>0.5%	>0.5%	>0.5%
Holy basil oil	>0.5%	>0.5%	>0.5%	>0.5%	>0.5%

Table 2. Minimum bactericidal concentration (%v/v) of essential oils against tested microorganisms

	Minimum bactericidal concentration (%v/v)				
	B. subtilis	*Brevibacterium* spp.	*M. luteus*	*S. epidermidis*	*S. aureus*
Lemongrass oil	0.0625%	0.25%	0.0625%	>0.5%	0.125%
Clove oil	0.125%	>0.5%	>0.5%	>0.5%	>0.5%
Cinnamon leaf oil	0.5%	>0.5%	>0.5%	>0.5%	>0.5%
Sweet basil oil	ND	0.5%	ND	ND	ND
Tumeric oil	>0.5%	ND	ND	ND	ND
Kaffir lime oil	ND	ND	ND	>0.5%	ND
Galanga oil	ND	ND	ND	ND	ND
Holy basil oil	ND	ND	ND	ND	ND

ND; not determined due to MICs >0.5% v/v

Brevibacterium spp. and *Micrococcus* spp. are bacteria living on the skin and they can cause a unique smell. However, there were a few studies of essential oils and very rare report on Thai essential oils against these bacteria. Van Vuuren, SF, et al., reported MICs of kanuka oil (*Kunzea ericoides*) and manuka oil (*Leptospermum scoparium*) against *Brevibacterium* spp. which were equal or less than 1 mg/ml[16]. In this study, MICs of essential oils against *Brevibacterium* spp. and *M. luteus* were determined. Lemongrass oil demonstrated the most potent agent against these bacteria. Orchard A, et al., also mentioned that lemongrass oil was one of promising essential oils against *S. aureus*[8]. Naik MI, et al., also demonstrated MICs of lemongrass oil against some pathogenic bacteria included *Bacillus* spp. and *S. aureus* which were about 0.03 to 0.06% v/v[17]. From our results, lemongrass oil possessed the highest bacteriostatic and bactericidal activities against all tested bacteria. Clove oil and cinnamon leaf oil were ranked as the second and third effective agents, respectively. Hence, lemongrass oil was selected for further study.

3.2. Compatibility study of essential oils and surfactants

To prepare self-emulsifying formulation, the suitable surfactant is a key of success for the formulation development. According to the antimicrobial assay of essential oils, lemongrass oil was used in this study. Four surfactants were selected to study the compatibility with lemongrass oil. After mixing each surfactant, the transparency of mixture was evaluated by observing the letter through the mixture as illustrated in Figure 1 and then scored as ++, + or – indicating very clear, slightly clear and turbid, respectively. The results showed that no phase separation was observed for all lemongrasss oil/surfactant mixtures. As summarized in Table 3, Tween 20 and Span 20 gave the clear mixture at all ratios. The mixture ratio at 5:5 showed the clearest solution. Therefore, Tween 20 and Span 20 were chosen for the formulation development at the lemongrass oil/surfactant ratio of 5:5.

Figure 1. Naked eyes observation of clarity/turbidity of lemongrass oil/surfactant mixture.

Table 3. Transparency results of the mixture of lemongrass oil and various surfactants

Lemongrass oil:surfactant ratio	Tween 20	Solutol HS 15	PEG 400	Span 20
1:9	+	-	-	+
5:5	++	+	-	++
9:1	+	++	++	+

++, + and – denote very clear, slightly clear and turbid appearance.

3.3. Self-emulsifying emulsion formulation

Self-emulsying emulsion is a system that contains oil and surfactant/co-surfactant. When gently mixing with water, fine emulsions will be spontaneously formed[14]. Self-emulsifying emulsions are normally formed with a droplet size between 100-300 nm whereas self-emulsifying microemulsions are produced with transparent characteristics with a droplet size of less than 50 nm[14, 18, 19]. From the compatibility results, two formulations consisting of lemongrass oil and Tween 20 or Span 20 were diluted with water by 160 folds on the basis of the purpose of foot bath formulation. However, the formed emulsions were unstable with subsequent phase separation (data not shown). In general,

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