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Antibacterial Activity of an Effective Spice Essential Oil Formulated in Foot Deodorant Gel against *Bacillus subtilis*

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Abstract: Skin bacterial flora, namely *Staphylococcus epidermidis,* are able to metabolise sweat that hence leads to foot odor. Moreover, *Bacillus subtilis* was found in the plantar skin of subjects possessing strong foot odor. The synthetic antibacterial agent generally used in various foot deodorant formulations is triclosan which tends to cause bacterial tolerance. To avoid that shortcoming, researchers in this study developed a natural foot deodorant gel from essential oils. Previous research of our group revealed that cinnamon oil showed a higher antibacterial activity against *S. epidermidis* than the essential oils obtained from kaffir lime, lemongrass, sweet basil, galanga and ginger. In the present study, the susceptibility of *B. subtilis* to the mentioned essential oils was determined and the results showed that cinnamon oil possessed the highest activity. Foot deodorant gel containing cinnamon oil was formulated and studied for its biological stability for 90 days at accelerated conditions. The lethal effect of the cinnamon oil gel exposed to *B. subtilis* for 1 h, was studied at day 0, 15, 30, 60 and 90. It was found that on all sampling days, cinnamon oil gel could decrease by at least 90% the initial bacterial population after 1 h of contact time. In conclusion, cinnamon oil foot deodorant gel demonstrated a good ability to decrease the bacteria involved in strong foot odor. The cinnamon oil foot deodorant gel might be an alternative cosmetic for people who have strong foot odor.

Key words: Cinnamon oil, foot odor, deodorant gel, antibacterial activity, *Bacillus subtilis*

INTRODUCTION

Foot odor has been characterized as a health problem. Its etiology attributes to degradation of the leucine found in sweat by the skin's normal bacteria flora, namely, *Staphylococcus epidermidis* which is the most Staphylococcal species investigated on glabrous skin (Rath *et al*., 2001; Troccaz *et al*., 2009). The major produced metabolites are short chain fatty acids including isovaleric acid, acetic acid, butyric acid and isobutyric acid which derived to foot odor (Ara *et al.,* 2006; Caroprese *et al*., 2009). Moreover, *Bacillus subtilis* is also found in the plantar skin of people having strong foot odor (Ara *et al*., 2006). Some of these people rely on deodorant to eliminate body odors such as axillary and foot odor. Deodorant is a type of cosmetic used to eliminate these body odors. Numerous dosage forms of foot deodorant have been distributed to the marketplace such as sprays (Kalavala *et al*., 2007) and creams (Caroprese *et al*., 2009). The antibacterial agent widely used in these various types of antibacterial cosmetics is triclosan. However, triclosan is a synthetic biocide which tends to cause bacterial tolerance (Cottell *et al*., 2009). As an alternative, researchers have explored natural antibacterials and subsequently reported on the antibacterial activity of essential oils from *Citrus hystrix* DC. (kaffir lime) fruit peel, *Cymbopogon citratus* Stapf. (lemongrass) grass, *Cinnamomum zeylanicum* Nees (cinnamon) bark, *Ocimum basilicum* L. (sweet basil) leaves, *Alpinia galanga* (L.) Willd. (galanga) and *Zingiber officinale* Rosc (ginger) rhizome (Inouye *et al*., 2001; Lertsatitthanakorn *et al*., 2006; Luangnarumitchai *et al*., 2007; Chanthaphon *et al*., 2008; Wannissorn *et al*., 2009). In our previous study, the susceptibility of *S. epidermidis* to these mentioned essential oils was determined. It was found that cinnamon oil showed the highest activity against *S. epidermidis* while kaffir lime oil and lemongrass oil revealed the second and third highest potencies, respectively (Chimmalee and Lertsatitthanakorn, 2010). The purpose of this study is to determine the antibacterial activity of these essential oils against *B. subtilis* by the broth

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microdilution method. Foot deodorant gel containing the oil most active against both *B. subtilis* and *S. epidermidis* was formulated and studied for its biological stability for 90 days. Hopefully, this cosmetic preparation might serve as an alternative foot deodorant for people with strong foot odor.

MATERIALS AND METHODS

Materials: Essential oils from *Citrus hystrix* DC. (kaffir lime) fruit peel, *Cymbopogon citratus* Stapf. (lemongrass) grass, *Cinnamomum zeylanicum* Nees (cinnamon) bark, *Ocimum basilicum* L. (sweet basil) leaves, *Alpinia galanga* (L.) Willd. (galanga) and *Zingiber officinale* Rosc (ginger) rhizome were purchased from Thai China Flavors and Fragrances Industry Co. (Thailand). Mueller-Hinton agar and Mueller-Hinton broth were provided by Himedia (India). Dimethyl sulfoxide was purchased from Analar (UK). Glycerin, carbomer 937, sodium chloride, propylene glycol, triethanolamine, polysorbate 80, methyl paraben, propyl paraben and lavender oil were purchased from local suppliers in Thailand.

Culture and growth condition: *B. subtilis* DMST 15896 strain was obtained from the Department of Medical Science, Ministry of Public Health, Thailand. A culture was maintained and grown in Mueller-Hinton broth at 37°C.

Determination of MIC and MBC values of the six essential oils against *B. subtilis*: A broth microdilution method was used to determine the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of the six essential oils in the same manner of the methods used by Lertsatitthanakorn *et al.* (2006) and Tarawneh *et al.* (2010). Muller-Hilton broth was added with 10% dimethyl sulfoxide to dissolve the oils. Fifty microliters of two folded dilutions of each oil sample were prepared in a 96-well plate. Fifty microliters of *B. subtilis* culture were added to each well to make a final concentration of approximately 10^8 CFU mL^{-1}. In each test, *B. subtilis* in Mueller-Hinton broth and Mueller-Hinton broth alone were used as a positive and negative growth control, respectively. The plates were then incubated at 37°C for 24 h before the MICs were determined. The MIC value was defined as the lowest concentration of the oil inhibiting visible growth of bacteria. Ten microliters of broth were removed from each well and spotted onto Mueller-Hinton agar to determine the MBC value. After incubation at 37°C for 24 h, the number of surviving *B. subtilis* was counted. The lowest concentration, where less than 0.1% of the initial inoculum survived, was defined as the MBC value. Each experiment was performed in triplicate.

Determination of the lethal effect of the selected essential oil against *B. subtilis*: The lethal effect determination was modified from the method previously described by Lertsatitthanakorn *et al.* (2010). A suspension of 0.1 mL of *B. subtilis*, approximately 10^8 CFU mL^{-1}, was added to 0.9 mL solution of the selected essential oil. The oil solution was composed of 10% dimethyl sulfoxide in Mueller-Hinton broth mixed with the selected oil to give the two desired concentrations of the oil. A 0.9 mL solution of 10% dimethyl sulfoxide in Mueller-Hinton broth mixed with a 0.1 mL suspension of *B. subtilis*, approximately 10^8 CFU mL^{-1}, was used as control. Two 0.1 mL samples and one 0.1 mL control were stirred carefully and transferred to three separate 0.9 mL tubes of normal saline solution at 0, 10, 20, 30 and 60 min. The samples were serially 10-fold diluted in normal saline solution and 100 μL of each sample were spread on Mueller-Hinton agar for viable counting. The experiment was performed in triplicate. Results are presented as time-log survivor's curves with bars representing the standard deviation.

Formulation of foot deodorant gel containing the selected essential oil: Various formulations of hydrogel containing a suitable concentration of the selected oil were prepared using carbomer as a gelling agent. To overcome the spicy odor of the selected essential oil, various perfume oils such as lavender and jasmine were added to the preparation. The foot deodorant gel with the best odor was chosen for determination of its physical stability using the freeze-thaw cycling method. The freeze-thaw cycling method consisted of keeping the selected deodorant at 4°C for 24 h and at 45°C for 24 h. The time frame for one complete cycle was two days; ten days was allocated for 5 cycles. Color, odor, pH and feeling, after applying deodorant gel on the foot, were recorded before the first cycle and after the fifth cycle. The deodorant gel (Table 3) that was selected was determined following the steps outlined in the next section.

Biological stability study of foot deodorant gel containing the selected essential oil against *B. subtilis*: The developed deodorant gel was stored in two separate environments for 90 days: room temperature with natural light and 45°C without light. At day 0, 15, 30, 60 and 90, the biological stability of the deodorant gel was studied by lethal effect determination as previously described by Lertsatitthanakorn *et al.* (2008). A bacterial suspension of

0.1 mL (approximately 10^8 CFU mL^{-1}) was added to 0.9 mL of the deodorant gel and mixed well in a vortex mixer. The samples were kept at 37°C. A sample of 0.1 mL was collected for serial 10-fold dilution at 0 and 1 h of contact time. The sample was then placed on Mueller-Hinton agar and counted for survival bacteria after 24 h incubation. The results were presented as log reduction of bacteria after 1 h exposure (log survivors of bacteria at 0 min contact time - log survivors of bacteria at 1 h contact time) and the storage time.

Data analysis: SPSS 11.5 for windows was used to perform statistical analysis. The statistical difference of the biological stability, in term of *B. subtilis* reduction ability, between the foot deodorant gel containing the selected oil stored at room temperature with natural light and the one kept at 45°C without light was analyzed by Student t-test. The p-value of less than 0.05 was considered statistically significant.

RESULTS

Susceptibility of *B. subtilis* to six essential oils: MIC and MBC values of six essential oils against *B. subtilis* are shown in Table 1. Cinnamon oil exhibited the highest antibacterial activity to *B. subtilis* with the lowest minimum inhibitory concentration (MIC) at 0.049 μL mL^{-1}. However, the Minimum Bactericidal Concentration (MBC) of all tested oils to *B. subtilis* was 100 or more than 100 μL mL^{-1}. Lemongrass and kaffir lime oils showed the second and third highest activity to *B. subtilis*, respectively. Therefore, the susceptibility of *B. subtilis* to the six essential oils showed a similar pattern as that of *S. epidermidis* obtained from our previous study. In that study, among the six essential oils, cinnamon oil exhibited the lowest MIC and MBC against *S. epidermidis* at the same range of 0.391-1.562 μL mL^{-1} (Chimmalee and Lertsatitthanakorn, 2010). Notably, cinnamon oil was the most active oil against both bacterial strains involved in foot odor and it will be scrutinized in the following section.

The lethal effect of cinnamon oil against *B. subtilis*: The lethal effect of cinnamon oil against the tested bacteria was measured to determine a suitable concentration of oil for foot deodorant preparation. According to our previous work, 3.125 and 9.375 μL mL^{-1} of cinnamon oil could decrease 1.5 log and 3 log of initial *S. epidermidis* population within 1 h, respectively (Chimmalee and Lertsatitthanakorn, 2010). Therefore, the desired concentrations for studying the lethal effect of cinnamon oil against *B. subtilis* were 3.125 and 9.375 μL mL^{-1}. As shown in Figure 1 and Table 2, both concentrations of cinnamon oil were able to reduce *B. subtilis* population rapidly. Because, the initial bacterial count was approximate 10^8 CFU mL^{-1} and reduced to less than 10^2 CFU mL^{-1} within 10 min after cinnamon oil exposure. By contrast, the solvent could not decrease *B. subtilis* population and contributed to the "control" curve in Fig. 1. Therefore, both concentrations of cinnamon oil were able to decrease at least 6 log of the initial population of *B. subtilis* within 10 min and this lethal effect was constant for 1 h of contact time. Therefore, 3.125 and 9.375 μL mL^{-1} of cinnamon oil were selected as suitable concentrations to decrease the population of *B. subtilis*. The gel base however, retards the release of the essential oil, so the higher concentration (9.375 μL mL^{-1}) was chosen to incorporate into a suitable gel base.

Table 1: MIC and MBC values of six essential oils against *B. subtilis*

Essential oils	MIC (μL mL^{-1})	MBC (μL mL^{-1})
Ginger	>100	>100
Galanga	>100	>100
Lemongrass	0.391-1.562	>100
Sweet basil	100	≥100
Kaffir lime	50 -100	≥100
Cinnamon	0.049	>100

Formulation of foot deodorant gel containing cinnamon oil: An amount of 9.375 μL mL^{-1} of cinnamon oil was incorporated into a suitable hydrogel base for foot application. Cinnamon oil foot deodorant gel using lavender oil as a perfume produced the most favorable odor and was chosen for further study (Table 3). To prepare the gel, 2 g of carbomer 937 was dispersed in

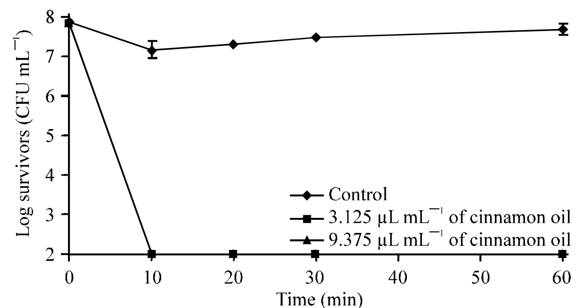

Fig. 1: Time-killing curves of cinnamon oil on *B. subtilis* Mean±SD, n = 3

Table 2: Log reduction of initial *B. subtilis* population after 1 h exposure with cinnamon oil

Concentration of cinnamon oil	Log reduction of *B. subtilis*
3.125 (μL mL^{-1})	≥6
9.375 (μL mL^{-1})	≥6

Table 3: Ingredients of the selected cinnamon oil foot deodorant gel

Ingredients	Quantity
Carbomer 937	2 g
Glycerin	4 g
Triethanolamine	0.12 g
Polysorbate 80	2 g
Paraben concentrated*	1 g
Cinnamon oil	937.5 μL
Lavender oil	0.44 g
Deionized water to	100 mL

*Paraben concentrated consisted of 10 g methyl paraben and 2 g propyl paraben dissolved in 100 mL propylene glycol

Table 4: Physical properties of the selected cinnamon oil foot deodorant gel before the first cycle and after the fifth cycle of freeze-thaw cycling storage

Physical properties	Before the first cycle	After the fifth cycle
Color	Opaque white	Opaque white
Odor	Mild lavender	Mild lavender
pH*	5.04±0.01	5.02±0.02
Feeling after applying on the foot	Good spreading	Good spreading

*pH of the foot deodorant gel was measured in triplicate by pH meter

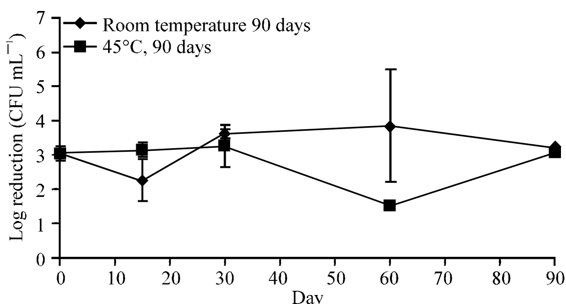

Fig. 2: Biological stability of cinnamon oil foot deodorant gel expressed as log reduction of *B. subtilis* after 1 h exposure with the gel, Mean±SD, n = 3

80 mL of deionized water and continuously stirred. 0.12 g of triethanolamine was used to neutralize carbomer until a clear gel base was achieved. Glycerin (4 g), polysorbate 80 (2 g) and 937.5 μL of cinnamon oil were then added and mixed. Paraben concentrated (1 g) and lavender oil (0.44 g) were added and mixed well. Finally, the volume of the gel was adjusted to 100 mL with deionized water. The physical stability of the cinnamon oil deodorant gel was studied using a freeze-thaw cycling method. The results in Table 4 revealed that the selected cinnamon oil foot deodorant gel possessed a physical stability. Because, the physical properties of the gel including color, odor and feeling after applying on the foot, did not change after the fifth cycle of freeze-thaw cycling storage. On the one hand, the pH of the gel was rather constant due to a minute changing from 5.04 (before the first cycle) to 5.02 (after the fifth cycle).

Biological stability study of the selected cinnamon oil foot deodorant gel against *B. subtilis*: As shown in Fig. 2, the log reduction of the tested bacteria exposed to cinnamon oil deodorant gel was more than 90% (1 log) for all sampling times. The bacterial reduction ability between the gel kept at room temperature with natural light and the gel kept at 45°C without light was not statistically significant different ($p>0.05$).

DISCUSSION

Cinnamon oil exhibited the highest antibacterial activity against the bacteria (*B. subtilis*) found in the plantar skin of subjects having strong foot odor. The MIC value of cinnamon oil to *B. subtilis* was rather low and compared well with the work of Prabuseenivasan *et al*. (2006). In our previous research, the major components in cinnamon oil, determined by Gas Chromatography-Mass Spectrometry, were cinnamaldehyde (32.66%) and eugenol (36.49%) respectively (Chimmalee and Lertsatitthanakorn, 2010). In the present research, it was suggested that the prominent antibacterial activity of cinnamon oil against *B. subtilis* might be attributable to both of these major constituents. The previous antibacterial activity study of cinnamon leaf and bark essential oils and their components clearly supported our results. Since, Singh *et al*. (2007) found that E-cinnamaldehyde and eugenol were the main components of cinnamon bark oil and cinnamon leaf oil, respectively. They determined antibacterial activity of these chemicals against six pathogenic bacteria including *B. subtilis* by agar well diffusion method. The results of anti- *B. subtilis* demonstrated that E-cinnamaldehyde showed a comparable inhibition zone with that of cinnamon bark oil at the same concentration. On the one hand, eugenol revealed a comparable zone with that of cinnamon leaf oil. Lu *et al*. (2011) found that cinnamon bark oil, rich in trans-cinnamaldehyde, showed a strong antibacterial activity against *B. subtilis* with the low MIC of 0.2 μL mL^{-1}. The essential oil of *Ocimum basilicum* (basil) containing 62.60% of eugenol also showed antibacterial activity against *B. subtilis* with the low MIC of 0.625 μL mL^{-1} (Lv *et al*., 2011). Cinnamaldehyde revealed a prominent antibacterial activity against *B. subtilis* by disc diffusion method and it showed a higher activity than benzoic acid at the same concentration (Wei *et al*., 2011). Moreover, cinnamaldehyde and eugenol revealed a potent antimicrobial activity to *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Staphylococcus aureus*, *Helicobacter pylori* and *Escherichia coli* (Ali *et al*., 2005; Baskaran *et al*., 2009; Pei *et al*., 2009). Matan (2007) also found that cinnamaldehyde and eugenol possessed antifungal activity against *Aspergillus niger* by disc diffusion assay. In addition, when using various skin sensitization

potency assay methods, cinnamaldehyde was classified as a mild to moderate contact allergen while eugenol was classified as a mild allergen (Kimber *et al*., 2003). Therefore, the low concentration of 9.375 $\mu L\ mL^{-1}$ of cinnamon oil added to the deodorant gel in the present study translates into a safe non-irritant concentration of cinnamaldehyde and eugenol.

Cinnamon oil foot deodorant gel stored at both accelerated conditions showed less *B. subtilis* reduction than pure cinnamon oil. A time killing assay of pure cinnamon oil revealed that the oil was able to reduce *B. subtilis* population rapidly, at least 6 log of the initial population, within 1 h. By contrast, the *B. subtilis* reduction ability of cinnamon oil foot deodorant gel ranged between 1-4 log of the initial population after 1 h exposure. This diminution in the ability of the cinnamon oil deodorant gel to reduce the *B. subtilis* population might be attributed to the cinnamon oil deodorant gel's exposure to natural light and high temperature. The same phenomenon was occurred with citronella oil oleogel, an anti-acne preparation, performed in our previous researches. The oleogel containing 6.5% v/w of citronella oil which kept at 40°C for 120 days, could reduce less than 1.5 log of the initial *Propionibacterium acnes* population after 12 h exposure (Lertsatitthanakorn *et al*., 2008). By contrast, 5% v/v citronella oil could decrease about 6.5 log of the initial *P. acnes* population within 6 h (Lertsatitthanakorn *et al*., 2010). In addition, the retard effect of the gel base might lead to a slow release of cinnamon oil from the gel even before it is exposed to bacteria. Nevertheless, cinnamon oil foot deodorant gel still possesses satisfactory biological stability due to its ability to decrease at least 1 log (90%) of *B. subtilis* population over a 90 days storage period. To prove a foot malodor relief efficacy of the developed gel, clinical trial should be studied in people who possessing strong foot odor in the future.

CONCLUSION

Cinnamon oil demonstrated the highest antibacterial activity to *B. subtilis,* the skin bacteria involved in strong foot odor. Therefore, cinnamon oil was suitable to use as an antibacterial agent in foot deodorant preparations. The developed cinnamon oil foot deodorant gel possessed a good ability to decrease *B. subtilis* population throughout the gels' 90 days storage period at accelerated conditions. To extend its shelf-life and bacterial reduction ability, cinnamon oil gel should be protected from light and exposure to high temperature. The cinnamon oil foot deodorant gel can be developed as an alternative cosmetic for people who have strong foot odor.

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