Application of Propolis Extract and Geraniol in Antiseptic Hand Gel

Patree Kaewmanee¹ Sasitorn Tongchipkadee¹,³ Nophadon Luangpirom² and Chitsiri Rachtanapun¹,³

ABSTRACT

The effect of antimicrobial activity of antiseptic hand gel containing propolis and geraniol was evaluated. Log reduction of microorganisms (the initial number was about 5 log CFU/ml) of hand gels containing ethanolic extract of propolis and geraniol compared with ethanol hand gel as the active ingredient was done in phosphate buffer saline (in vitro). The hand gel with EEP and geraniol (EEPGer) showed the strongest antimicrobial activity against S. aureus and E. coli. Those cell counts reduced by 90% within 15 and 30 sec, respectively. For the in vivo, the swab test from 110 volunteer’ hands before and after using different hand gels including EEPGer, ethanol or triclosan (commercial) showed that the antibacterial activity against total plate count after using three hand gels were not significant difference (p ≤ 0.05). Therefore, the application of propolis and geraniol in antiseptic hand gel can be used as natural alternative to replace chemical substances.

Key words: propolis, ethanolic extract, geraniol, antimicrobial activity, antiseptic hand gel

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INTRODUCTION

Propolis is a resinous substance derived from plant sources which was collected by bees. It has broad spectrum antimicrobial activity against bacteria, fungi and virus (Stepanovic et al., 2003; Kaewmanee and Rachtanapun, 2011). In addition, propolis has found to possess antioxidant, antiulcer, anticancer and anti-inflammatory activities (Martos et al., 2008). Therefore, it has been widely used commercially on the market, as a useful ingredient for the applications in supplementary food, medicine and domestic goods; for example, toothpaste, soap, mouth rinse and spray. However, the application is limited due to its dark-brown color and astringent taste.

Geraniol is a commercially important acyclic monoterpenic alcohol occurring in the essential oils of several aromatic plants such as lemon grass, citronella, mint oil, rose oil, palmarosa oil and geranium (Chen and Viljoen, 2010). It appears as a clear to pale-yellow. It is one of the most important molecules in the flavor and fragrance industries and is a common ingredient in consumer products produced by these industries. In addition to its pleasant odor, geraniol possesses antimicrobial activity against bacteria and fungi (Friedman et al., 2002). Moreover, it is also found to have antioxidant, anticancer, anti-inflammatory and insecticide (Chen and Viljoen, 2010).

Furthermore, the antimicrobial activity of ethanolic extract of propolis (EEP) and geraniol showed significant antibacterial activities against S. aureus (MIC 1.0 and 0.08, respectively) and E. coli (MIC 7.0 and 0.5, respectively). In addition, the combination effect of EEP and geraniol showed synergistic effect against Gram-positive and Gram-negative bacteria and yeast, the strongest antimicrobial activity was at ratio 0.4:0.02 against S. aureus (Kaewmanee et al., 2012).

Antiseptic hand gel is the product for the removal or destruction of transient microorganisms and reduce resident flora. Antimicrobial ingredients used in hand gel are various such as alcohol, chlorhexidine gluconate, hexachlorophene, iodine and iodophors, para-chloro-meta-xylenol and triclosan (Larson, 1995); however, alcohol is the most commonly used in commercial products. It has excellent bactericidal activity against most vegetative Gram-positive and Gram-negative bacteria. Although it is not sporicidal, it acts against many fungi and virus including hepatitis B and HIV due to it probably derives the antimicrobial effect by denaturation of proteins. However, disadvantage of alcohol for skin is drying effect. Most marketed preparation contains 60-70% ethanol with the addition of moisturizing agents (Larson, 1995). Thus, it is interesting to combine beneficial potentials of propolis and geraniol in order to enhance antimicrobial activity, alcohol reduction and improve the odor and color for application in hand gel product.

Therefore, the objective of this study was to investigate the antimicrobial activity of ethanolic extracts of propolis (EEP) and geraniol to apply for antiseptic hand gel, by determining the log reduction of microorganisms for the in vitro and in vivo after using antiseptic hand gels.
MATERIALS AND METHODS

1. Bacterial strains and inoculum preparation
The tested bacteria namely Escherichia coli TISTR 780 and Staphylococcus aureus TISTR 1466 were obtained from Thailand Institute of Scientific and Technological Research (TISTR). They were kept on tryptic soy agar (TSA; Merck, Darmstadt, Germany) slant at 4°C and cultured in 10 ml of tryptic soy broth (TSB; Merck) at 37°C for 2 successive 24 h and 18 h transfers before used.

2. Preparation of ethanolic extract of propolis
Dried propolis from Chiang Mai province was obtained from Heaven Herb Co., Ltd. (Pathumthani, Thailand). Propolis was mixed with 95% ethanol to obtain 20% (w/v) EEP. The ethanol was not evaporated.

3. Preparation of antiseptic hand gel
Antiseptic hand gel was prepared as modified from Faculty of Pharmacy, Mahidol University (2009). The following ingredients were used in Table 1. Carbopol 940 (Lubrizol Advanced Materials, Hong Kong) was dissolved and stirred in warm water (50°C) on hot plate with magnetic stirrer until gel was occurred. Then, absolute ethanol was mixed with gel, and added with EEP and geraniol (Thai-China Flavours and Fragrances Industry Co., Ltd., Thailand) for EEPGer formula. After that, triethanolamine was added and mixed thoroughly. The hand gels were kept in close lid container.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Ingredients</th>
<th>Carbopol 940</th>
<th>Triethanolamine</th>
<th>Absolute ethanol</th>
<th>Water</th>
<th>EEP</th>
<th>Geraniol</th>
</tr>
</thead>
<tbody>
<tr>
<td>E30</td>
<td></td>
<td>0.5 g</td>
<td>0.3 ml</td>
<td>30 ml</td>
<td>69.2 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E70</td>
<td></td>
<td>0.5 g</td>
<td>0.3 ml</td>
<td>70 ml</td>
<td>29.2 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EEPGer</td>
<td></td>
<td>0.5 g</td>
<td>0.3 ml</td>
<td>25.8 ml</td>
<td>69.2 ml</td>
<td>4 ml</td>
<td>0.2 ml</td>
</tr>
</tbody>
</table>

E30 - Hand gel containing 30% (v/v) ethanol; E70 - Hand gel containing 70% (v/v) ethanol; EEPGer - Hand gel containing EEP and geraniol

4. Antimicrobial assessment of antiseptic hand gel
In vitro antimicrobial activity of antiseptic hand gel was assessed in two strains of bacteria including S. aureus and E. coli. Sample solution was prepared by mixing 1 ml of cell suspension in 69 ml of phosphate buffer saline (PBS) (the initial number was about 5 log CFU/ml). The hand gel sample of 30 ml was added into the solution. Control solution was not added hand gel. The number of survival
microorganism was determined by plating on TSA every 0, 15, 30, 45 sec, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 min, plates were incubated for 24 h at 37°C.

*In vivo* antimicrobial activity of different antiseptic hand gels including natural (EEPGer) hand gel, 70% (v/v) ethanol hand gel and commercial hand gel, containing triclosan (Myseptic Mybacin; Greater Pharma Manufacturing Co., Ltd., Nakhon Pathom, Thailand) were determined by using swab test from 110 volunteer’ hands. One pump volume of hand gel samples (approximately 0.75 g) was applied on the palms of the volunteers and they were asked to rub the gel thoroughly until the palms became dry (about 45 sec). Before and after using antiseptic hand gels, each sterile cotton swab stick was used to swab on 5×5 cm² of volunteer’ palms. Total viable count was determined by spread plate technique on TSA and incubated for 24 h at 37°C.

**RESULTS AND DISCUSSION**

1. **Antimicrobial activity of hand gels**

Figure 1 shows antimicrobial activity of hand gels against *S. aureus* and *E. coli* over time for 10 min. EEPGer showed the strongest antimicrobial activity and followed by E70. The EEPGer reduced *S. aureus* and *E. coli* numbers by about 1 log (90%) within 15 and 30 sec, respectively (Table 2 and 3). Furthermore, it reduced both bacteria by 3 logs (99.9%) within 2 min. In contrast, hand gel containing 30% v/v ethanol (E30) had no antimicrobial activity.

![Figure 1](image1.png)

*Figure 1* Trends of survival numbers of *S. aureus* (a) and *E. coli* (b) in PBS after adding hand gels at 0, 15, 30, 45 sec, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 min.
Table 2 Mean log reduction with standard deviation of *S. aureus* in PBS containing different hand gels

<table>
<thead>
<tr>
<th>Time</th>
<th>Mean log reduction (log CFU/ml)</th>
<th>E30</th>
<th>E70</th>
<th>EEPGer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 sec</td>
<td>0.00±0.00^a</td>
<td>0.00±0.00^a</td>
<td>0.00±0.00^a</td>
<td></td>
</tr>
<tr>
<td>15 sec</td>
<td>0.08±0.31^c</td>
<td>1.12±0.22^b</td>
<td>1.93±0.25^a</td>
<td></td>
</tr>
<tr>
<td>30 sec</td>
<td>0.04±0.25^b</td>
<td>2.09±0.11^a</td>
<td>2.26±0.21^a</td>
<td></td>
</tr>
<tr>
<td>45 sec</td>
<td>0.14±0.21^b</td>
<td>2.27±0.07^a</td>
<td>2.34±0.14^a</td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>0.16±0.49^b</td>
<td>2.36±0.38^a</td>
<td>2.62±0.20^a</td>
<td></td>
</tr>
<tr>
<td>2 min</td>
<td>0.08±0.06^b</td>
<td>2.88±0.04^a</td>
<td>3.03±0.13^a</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>0.14±0.24^b</td>
<td>4.06±0.93^a</td>
<td>4.81±0.42^a</td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>0.18±0.45^b</td>
<td>5.26±0.23^a</td>
<td>5.21±0.16^a</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a-c}\) Means within the same row followed by different letters were significantly different (p ≤ 0.05).

Comparing among tested microorganisms, *E. coli* was more resistant to antiseptic hand gel than *S. aureus*. The result was similar to Kaewmanee et al. (2012) who reported the combination antimicrobial activity of EEP and geraniol against *E. coli* (MICs of the combination was 4.0:0.1) and *S. aureus* (MICs of the combination was 0.4:0.02) and also report that *E. coli* was more resistant to EEP.
and geraniol than S. aureus because of a complicated structure of cell wall in Gram-negative bacteria of E. coli.

Furthermore, propolis alone had antimicrobial activity against bacteria and fungi. This result was supported by an earlier study on EEP against 39 microorganisms by using agar dilution method, showed significant antimicrobial activities against Gram-positive bacteria (MIC 0.078-1.25% of EEP) and yeasts (0.16-1.25%), while Gram-negative bacteria were less susceptible (1.25->5%) (Stepanovic et al., 2003). The most important active constituents of propolis are phenolic compounds, especially flavonoids and phenolic acids such as quercetin, apigenine, galangine and caffeic acid. These compounds were tested upon E. coli ATCC 25922 by plate culture method. They showed MIC 14.3±6 mg soluble compounds/ml of the most active (Tosi et al., 2007). In addition, antimicrobial activities of flavonoids have been reported due to their inhibitory action on DNA and RNA synthesis (Mori et al., 1987). Later, Mirzoeva et al. (1997) showed that one of flavonoids constituent, quercetin, may decrease capacity for ATP synthesis, membrane transport and motility of bacteria.

Friedman et al. (2002) also reported that geraniol was the most bactericidal active against E. coli (with a bactericidal activity value of BA$_{50}$ 0.15), L. monocytogenes and S. enterica. As a monoterpenne member, geraniol had antimicrobial inhibitory effects through the interaction with membrane structure and function due to their lipophilic and solubility properties (Rasoul et al., 2012).

2. Antimicrobial activity of hand gels in vivo

The antimicrobial efficacy of different antiseptic hand gels is shown in Table 4. The efficacy of EEPGer, E70 and commercial hand gel containing ethanol and triclosan were not significant difference (p ≤ 0.05). These antiseptic hand gels reduced total viable counts on volunteer' palms by 0.69-0.83 log CFU/25 cm$^2$ after applied for about 45 sec.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Active ingredients</th>
<th>Mean total viable counts (CFU/25 cm$^2$)</th>
<th>log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>EEPGer</td>
<td>4% EEP, 0.2% Geraniol, 25.8% Ethanol</td>
<td>2.63 ± 0.64$^{aA}$</td>
<td>1.86 ± 0.62$^{bB}$</td>
</tr>
<tr>
<td>E70</td>
<td>70% Ethanol</td>
<td>2.17 ± 0.61$^{aA}$</td>
<td>1.34 ± 0.49$^{bB}$</td>
</tr>
<tr>
<td>Commercial</td>
<td>Ethanol, Triclosan</td>
<td>2.40 ± 0.55$^{aB}$</td>
<td>1.71 ± 0.53$^{bB}$</td>
</tr>
</tbody>
</table>

$^{a-b}$ Means within the same column followed by different letters were significantly different (p ≤ 0.05).
$^{A-B}$ Means within the same row followed by different letters were significantly different (p ≤ 0.05).
For the appearance of hand gels, EEPGer was a viscous yellow aroma gel and slow evaporation after rubbed on hand. E70 and commercial had similar appearance; they were less viscous gel with clear, alcohol odor and dry faster after rubbed on hand. However, disadvantage of ethanol were fast evaporation, skin drying, flammable and consequently must be stored carefully (Larson, 1995). Triclosan, a synthetic broad spectrum antimicrobial agent, may cause photoallergic contact dermatitis which occurs when the part of the skin exposed to sunlight (Glaser, 2004). Furthermore, it reacts with chorine to form chloroform, a carcinogenic substance, which may be absorbed through the skin. The safety level of triclosan is not over 0.3% (Food and Drug Administration, 2012). While, EEPGer had viscous appearance and could maintain moisture and antimicrobial active compounds for a longer time comparing to E70 and commercial hand gels. Furthermore, propolis contains vitamin C and E which beneficial for skin care (Tikhonov and Mamontova, 1987).

Therefore, the hand gel containing natural antimicrobial agents including propolis and geraniol was found to be effective and non skin drying when compared with alcohol. Moreover, considering on price of product basis on 100 ml, EEPGer (15 bahts) is cheaper than E70 (30 bahts) and commercial (60 bahts).

CONCLUSION

Considering the results, it can be concluded that application of EEP and geraniol in antiseptic hand gel had significant antibacterial potential against *S. aureus* and *E. coli* tested *in vitro* and it had antimicrobial activity against total viable counts *in vivo*. Thus, combination of propolis and geraniol has a potential to use as natural antimicrobials for application in antiseptic hand gel to replace chemical substances.

ACKNOWLEDGEMENTS

This research was financially supported by Thailand Research Fund (TRF) Science and Technology, and Heaven Herb Co., Ltd. – Master Research Grants under the projects sector production linked with the Thailand Research Fund (TRF)-Industry annual 2553 (MRG-WI535S017).

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