Effect of an essential oil blend of citronella, lemongrass, and patchouli on acne-causing bacteria

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Received: 20 March 2019; Revised: 28 June 2019; Accepted: 21 July 2019

Abstract

Currently there is increasing interest in antibacterial effects of blended essential oils. Our study aimed to determine a suitable ratio of citronella (C), lemongrass (L), and patchouli (P) essential oils, which could be used to combat the acne causing bacteria Propionibacterium acnes and Staphylococcus epidermidis. MIC values of the oil blends were estimated by broth dilution method. Effects of blended oil against P. acnes and S. epidermidis were estimated from the fraction inhibitory concentration index (FICI). Patchouli oil exhibited the strongest activities against both microorganisms, but excessive patchouli oil in the blend was antagonistic to effects against both microorganisms. A suitable blend ratio was C:L:P (1:2:1) by weight, which showed the strongest antibacterial activities against P. acnes and S. epidermidis (MIC = 0.3125 and 0.625 mg/ml, respectively), with synergistic effects (FICI = 0.09 and 0.20, respectively). The major constituents in the oil blend were citronellal, citral, and patchouli alcohol, provided by each type of oil.

Keywords: citronella oil, lemongrass oil, patchouli oil, Propionibacterium acnes, Staphylococcus epidermidis

1. Introduction

Propionibacterium acnes and Staphylococcus epidermidis are important pathogens causing acne vulgaris that affects almost every person at least once during their lifetime. P. acnes is an anaerobic bacterium that resides underneath the skin surface and inhabits the androgen stimulated sebaceous follicles. In contrast, S. epidermidis is an aerobic organism involved in the superficial infection within the sebaceous unit. The hyperactivity of resident microflora populations is one stage of acne pathology, and both of the microorganisms are targets for anti-acne drugs (Dawson & Dellavalle, 2013; Kurokawa et al., 2009; Thiboutot et al., 2009). Increased bacterial resistances due to long-term and frequent uses of antibacterial drugs drive the search for novel compounds from natural sources (Sheetal & Singh, 2011; Sinha, Srivastava, Mishra, & Yadav, 2014).

Several reports have indicated activities of essential oils against various kinds of bacteria, including P. acnes and S. epidermidis (Andradea, Barbosaa, Probst & Júnior, 2014; Chouhan, Sharma, & Guleria, 2017; Sinha et al., 2014). Recently, effects of combining essential oils have been studied. Mixtures of essential oils have produced various effects on microorganism, with synergistic, additive, or antagonistic effects (Bassolé et al., 2010; Chouhan et al., 2017; Fu et al., 2007; Goñi et al., 2009; Gutierrez, Barry-Ryan, & Bourke, 2008; Tadtong et al., 2012; Wagnera & Ulrich-Merzenich, 2009; ). These effects might be caused by interactions between the constituents in the essential oils, or among different essential oils (Sinha et al., 2014; Wagnera & Ulrich-Merzenich, 2009). The interactions in each combination of essential oils are estimated with a checkerboard assay, which

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is used to explore the potency of combinations of antibacterial components in comparison to their individual activities. This comparison is then represented in terms of the fractional inhibitory concentration index (FICI), which is the sum of fraction inhibitory concentrations (FICs). The FIC is the ratio of essential oil concentration to its own MIC. The effect is categorized as synergistic, indifferent (or no interaction), or antagonist, according to the FICI is 0.5 or less, more than 0.5-4.0, or more than 4, respectively (Odds, 2003).

Citronella (Cymbopogon nardus), lemongrass (Cymbopogon citratus), and patchouli (Pogostemon cablin) oils were obtained from hydrodistillation. The most abundant compounds of citronella oil are citronellal, citronellol, and geraniol (Ganjewala, 2009; Nakahara, Alzoreky, Yoshihashi, Nguyen, & Trakoontivakorn, 2013; Wei & Wee, 2013). Lemongrass oil is primarily composed of citral and β-myrcene (Hamad, Nuritasari, & Hartanti, 2017; Tajadin, Ahmad, Rose nani, Azimah, & Munirah, 2012). Patchouli oil consisted of patchouli alcohol, α-bulnesene, and α-guaiane (Bunrathep, Lockwood, Songsak, & Ruangrungsi, 2006). These essential oils and their active constituents have previously been reported as active against microorganisms, including P. acnes and S. epidermidis (Chouhan et al., 2017; Luangnarumitchai, Lamlerton, & Tiyaboonchai, 2007; Onawunmi, 1989; Onawunmi, 2011; Wan et al., 2016; Yang, Zhang, Yang, & Lui, 2013). However, effects of blends of these oil preparations have not been reported.

This study aimed to evaluate the effects of these essential oils in various blend ratios, and to determine suitable mixture proportions with synergistic antibacterial effects against P. acnes and S. epidermidis. The results could then be applied in cosmetic or pharmaceutical products.

2. Materials and Methods

2.1 Essential oils and chemicals

Pure essential oils of Cymbopogon nardus leaf (citronella oil), Cymbopogon citratus leaf (lemongrass oil), and Pogostemon cablin (patchouli oil) were obtained by steam distillation of known constituents purchased from Thai-Chinese Flavors and Fragrances Industry Co. Thailand. Standard citronella, lemongrass, and patchouli alcohol were purchased from Sigma-Aldrich, Inc. (St. Louis, Mo, USA). All the components were kept in amber glass bottles with tightly fitting lids, which were stored at 4°C prior to use.

2.2 Bacterial strains

The test microorganisms used in this experiment were Propionibacterium acnes DMST 14916 and Staphylococcus epidermidis TISTR 518. These bacteria were obtained from the Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University. P. acnes was incubated at 37°C in Brain Heart Infusion (BHI) agar for 48-72 h under anaerobic conditions, while S. epidermidis was cultured at 37°C in Mueller Hinton agar (MHA) for 24 h.

2.3 Blended oil preparation

Various ratios (by weight) of citronella oil, lemongrass oil, and patchouli oil were mixed. Briefly, the largest amount of an essential oil was pipetted into the vial first, followed by adding the minor components into the same vial. The oil blend was then homogenized and stored at 4°C prior to use.

2.4 Disc diffusion method

The antibacterial activity was evaluated with traditional antibiotic susceptibility testing, using the disc diffusion method as described by Bauer, Kirby, Sherris, & Turck (1966). The activity was determined by measuring the diameter of the zone of inhibition against the test microorganisms. Three independent experiments were performed and each experiment was run in triplicate. Sterile 6 mm paper discs were impregnated with 15 μl undiluted essential oil and deposited on the agar surface, with 1% clindamycin serving as the positive control.

2.5 Broth dilution method

The antibacterial effects of essential oils were evaluated by determining the minimum inhibitory concentration (MIC) with the broth dilution method, according to the modified protocol described by Gutierrez et al. (2008). Briefly, essential oils were dissolved in 5% DMSO, then diluted in media and tested over a range of concentrations from 0.3125-10,000 μg/ml against overnight broth cultures of bacteria grown to a concentration of 5x10^8 CFU/ml in liquid culture media. Clindamycin was used as a positive control. Microbial growth was determined by observable turbidity. The lowest concentration of essential oil that completely inhibited growth of a particular microorganism is the MIC. Three independent experiments were performed and each experiment was run in triplicate. To study the effects of essential oils in a blend, the MIC for blended oils was compared to the MICs of single oils.

2.6 FIC index analysis

Checkerboard assay was used to estimate the interactions of essential oils in blends. Fraction inhibitory concentration index (FICI) was derived from MIC of essential oil combination, with no visible growth of the test microorganism. This demonstrated that the combinations of agents could exert inhibitory effects that exceeded the sum of their effects singly. FICI was calculated as follows.

\[
FIC index = \sum \left( \frac{MIC of each essential oil combination}{MIC of essential oil alone} \right)
\]

The interpretation was considered synergistic for FIC index of 0.5 or less (FICI ≤ 0.5), indifferent (or no interaction) for FICI > 0.5-4.0, and antagonistic for FICI > 4.0 (Odds, 2003).
2.7 GC-MS analysis

Essential oil constituents were identified by use of Gas Chromatography-Mass Spectrometry (GC-MS) 7890A, 5975C MSD (Agilent Technologies). The column was Mega-5MS (5% phenyl, 95% methyl polysiloxane) (30 m X 0.25 mm. i.d; 0.25 μM); oven temperature was 60°C for 1 min., then increased to 240°C at a rate of 3°C/min; injector temperature 180°C; injection volume 1 μl; transfer temperature 290°C for 5 min, and the carrier gas was He (2 ml/min). MS parameters were as follows: EI mode, ionization voltage 70eV, ion source temperature 230°C, and scan range 40-650 amu. 

Compounds were analyzed by comparing the Kovats gas chromatographic retention indices of the peaks on the HP-5MS column with literature values, computer matching against the NIST 2011 database, and comparison of the fragmentation patterns of mass spectra with those reported in the literature (Adams, 2007; Davies, 1990).

3. Results and Discussion

3.1 Antibacterial activity

Antibacterial activities of individual oils and their major constituents against acne-causing bacteria were screened by the disc diffusion method. All of the oils exhibited promising inhibition zones against Propionibacterium acnes DMST 14916 and Staphylococcus epidermidis TISTR 518 with the diameters of inhibition values given in Table 1. The strongest antibacterial activities against P. acnes and S. epidermidis belonged to lemongrass oil and patchouli oil, respectively, these having the widest inhibitory clear zones.

The minimum inhibitory concentrations (MIC) of essential oils and their major constituents were determined by broth dilution method, with results given in Table 2. Among the single oils, patchouli oil exhibited the highest efficacy against both P. acnes and S. epidermidis with the lowest MICs in the range 1.25-2.5 mg/ml, followed by lemongrass oil and then citronella oil. Even though the inhibition zone of lemongrass oil was larger than that of patchouli oil, its MIC value was greater than that of patchouli oil. This might be due to its ability to diffuse from the paper disc and solubilize in agar. Citral, the major constituent in lemongrass oil, gave the highest efficacy against both P. acnes and S. epidermidis with MIC in the range 0.125-0.25 mg/ml, followed by patchouli alcohol and citronellal.

Table 1. Antibacterial activities of the selected essential oils by disc diffusion method.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inhibition zone ± SD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Citronella</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>17.20 ± 0.00</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>15.50 ± 0.70</td>
</tr>
</tbody>
</table>

1% Clindamycin was used as a positive control. The tests were done in triplicate

Previous reports have assessed the effects of combinations of essential oils on several microorganisms. The various ratios of the same constituents in essential oil blends tend to interact with each other, acting in additive, synergistic, and in a few cases antagonistic fashion (Bassoulé et al., 2010; Fu et al., 2007; Golli et al., 2009; Gutierrez et al., 2008; Tadtong et al., 2012). Various mixtures (w/w) of blended oils were prepared (as shown in Table 2) in order to study the effects of blended oil preparations and find a suitable ratio against both P. acnes and S. epidermidis. The oil blends exhibited antibacterial activity against P. acnes and S. epidermidis in the agar disc diffusion test, and their MIC values as determined by broth dilution method are summarized in Table 2.

The results indicate that the blend with citronella (C), lemongrass (L) and patchouli (P) oils in (1:2:1) proportions exhibited the strongest activity against P. acnes, followed by the blends C:L (1:1) and L:P (1:1) in this order. Surprisingly, all three blends had a dominant fraction of lemongrass oil, with citral as its active constituent. This suggests citral as apparently the most active constituent against P. acnes. On the other hand, the blends C:L:P (2:1:2) and (1:2:2) gave the lowest activities against P. acnes, but they showed the strongest activities against S. epidermidis.

In this experiment, all these oil blends exhibited synergy against S. epidermidis. The blends C:L:P (1:1:1), (2:1:1), (1:1:2), and (2:2:1) displayed stronger activity against S. epidermidis than against P. acnes. Considering patchouli oil, although it exhibited the strongest activity against P. acnes with the lowest MIC value, unfortunately increasing the fraction of patchouli oil as in C:L:P (1:1:2) led to lower activity than that of C:L:P (1:1:1).

According to previous reports, MIC values above 1000 μg/ml denote inactivity, so the blends C:P (1:1), C:L:P (1:1:1) and (2:1:1) would not be appropriate for acne treatment, since their MICs were >1000 μg/ml against both P. acnes and S. epidermidis.

3.2 FICI index analysis

The fractional inhibitory concentration index (FICI) of each blended oil was calculated from its MICs against P. acnes & S. epidermidis. The results shown in Table 2 were interpreted as synergistic (FICI ≤ 0.5), no interaction (FICI > 0.5-4.0), or antagonistic (FICI > 4.0) (Odds, 2003).

All oil blends showed synergy against S. Epidermidis with the FICI values ranging from 0.07 to 0.35. The FICI of blends C:L:P (1:1:1), (2:1:1), and (1:2:1) against P. acnes were 4.33, 3.50, and 0.09, respectively, so the antibacterial effect against P. acnes would decrease with fraction of citronella oil, and this effect got stronger with larger fractions of lemongrass oil.

The synergistic effects against both P. acnes and S. epidermidis were present only for the mixtures C:L (1:1), L:P (1:1), and C:L:P (1:2:1). Considering the MIC values, although the blends C:L (1:1) and L:P (1:1) had similar MIC values against both P. acnes and S. epidermidis, the C:L (1:1) had more synergy than L:P (1:1) as the FICI value for C:L (1:1) is lower than that for L:P (1:1). Increasing the fraction of citronella oil or of lemongrass oil from the mixture of C:L:P (1:1:1) to (2:1:1), (1:2:1) or (2:2:1), showed increased synergy
Table 2. MICs of the essential oils and their major constituents against *P. acnes* and *S. epidermidis*, and FICI indexes of oil blends.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Blend oil* (w/w)</th>
<th>Citronella oil</th>
<th>Lemongrass oil</th>
<th>Patchouli oil</th>
<th>FICI <em>(Σ FIC)</em>**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alone</td>
<td>Combined</td>
<td>FIC</td>
<td>Alone</td>
</tr>
<tr>
<td><em>P. acnes</em></td>
<td>C.L (1:1)</td>
<td>1.25</td>
<td>12.5</td>
<td>0.625</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>C.P (1:1)</td>
<td>1.25</td>
<td>0.050</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>L.P (1:1)</td>
<td>NA</td>
<td>NA</td>
<td>0.625</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>C.L (1:1)</td>
<td>12.5</td>
<td>0.333</td>
<td>12.5</td>
<td>0.667</td>
</tr>
<tr>
<td></td>
<td>(2:1:1)</td>
<td>12.5</td>
<td>0.500</td>
<td>12.5</td>
<td>0.500</td>
</tr>
<tr>
<td></td>
<td>(1:2:1)</td>
<td>0.3125</td>
<td>0.006</td>
<td>0.3125</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>(1:1:2)</td>
<td>25</td>
<td>0.500</td>
<td>25</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>(2:2:1)</td>
<td>12.5</td>
<td>0.400</td>
<td>12.5</td>
<td>0.800</td>
</tr>
<tr>
<td></td>
<td>(2:1:2)</td>
<td>&gt;25</td>
<td>&gt;0.8</td>
<td>&gt;25</td>
<td>&gt;0.80</td>
</tr>
<tr>
<td></td>
<td>(1:2:2)</td>
<td>&gt;25</td>
<td>&gt;0.4</td>
<td>&gt;25</td>
<td>&gt;1.60</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>C.L (1:1)</td>
<td>1.25</td>
<td>0.025</td>
<td>0.25</td>
<td>0.625</td>
</tr>
<tr>
<td></td>
<td>C.P (1:1)</td>
<td>1.25</td>
<td>0.025</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>L.P (1:1)</td>
<td>NA</td>
<td>NA</td>
<td>0.625</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>C.L (1:1)</td>
<td>1.25</td>
<td>0.017</td>
<td>1.25</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>(2:1:1)</td>
<td>1.25</td>
<td>0.025</td>
<td>1.25</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>(1:2:1)</td>
<td>0.625</td>
<td>0.006</td>
<td>0.625</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>(1:1:2)</td>
<td>0.625</td>
<td>0.006</td>
<td>0.625</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>(2:2:1)</td>
<td>0.625</td>
<td>0.010</td>
<td>0.625</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>(2:1:2)</td>
<td>0.3125</td>
<td>0.005</td>
<td>0.3125</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>(1:2:2)</td>
<td>0.3125</td>
<td>0.005</td>
<td>0.3125</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*C* = citronella oil, *L* = lemongrass oil, *P* = patchouli oil, "Result interpreted as synergy (FICI ≤ 0.5), antagonist (FICI > 4), or no interaction (FICI > 0.5–4). The tests were done in triplicate. NA = not available.

3.3 Chemical compound identification

The chemical constituents in the selected oil mixture C.L:P (1:2:1) were identified by GC-MS. Shown in Table 3, twenty seven compounds (84.28%) in the blend were identified as 3 monoterpenes, 10 oxygenated monoterpenes, 10 sesquiterpenes, 3 oxygenated sesquiterpenes, and 1 phenylpropanoid. Patchouli alcohol (23.38%) was the main constituent, followed by geranial (trans-citral) (11.44%), α-bulnesene (10.09%), and neral (cis-citral) (9.83%). The GC-MS chromatogram for the oil blend is exhibited in Figure 1.

Citronellal, citral (neral & geranial), and patchouli alcohol are the active components in citronella, lemongrass, & patchouli oils, respectively, and these dominated in all the oil mixtures. These three compounds have been reported to have antimicrobial activities. Citral exhibited antimicrobial activity against *P. acnes* (Onawunmi, 1989; Onawunmi et al., 1984), similar to citronellal, geranial, citronellol in citronella oil (Nakahara et al., 2013; Tajidin et al., 2012), and patchouli alcohol (Luangnarumitchai et al., 2007; Wan et al., 2016). Thus, antibacterial activities against *P. acnes* and *S. Epidermidis* of this blend may be due to the activities of citronellal, citral, and patchouli alcohol. Consequently, these three compounds were chosen as chemical markers for quality control because they are the dominant component in this oil blend.

4. Conclusions

Citronella, lemongrass, and patchouli oils can be combined to an oil blend, and a suitable blend ratio is 1:2:1. This blend has promising antibacterial effects against *Pro-
Acknowledgements

The authors would like to acknowledge Research Institute of Rangsit University, Thailand, for financial support.

References


