RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2021.093

*ScienceAsia* 47 (2021): **690–697**

# Lemongrass essential oil enhances antibacterial activity of cephalexin against *Staphylococcus pseudintermedius* isolated from dogs with superficial pyoderma

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*Received 9 Apr 2021*
*Accepted 3 Aug 2021*

**ABSTRACT**: Superficial pyoderma in pets caused by *Staphylococcus pseudintermedius* is a common disease in veterinary medicine. Lemongrass essential oil has potent antibacterial effects, but studies of synergism of lemongrass essential oil with antibiotics are limited. This study examined the synergy of lemongrass essential oil with cephalexin against 7 *S. pseudintermedius* isolates obtained from dogs with superficial pyoderma by the checkerboard method and time-kill test. All isolates tested were sensitive to methicillin, and the MICs of cephalexin and lemongrass essential oil ranged from 1–4 and 780–1560 μg/ml, respectively. The checkerboard assay indicated that lemongrass essential oil had a partial synergistic effect with cephalexin; the concentration of cephalexin and lemongrass essential oil required to inhibit bacterial growth was reduced by 2–4 times. Time-kill assay revealed that the effects of cephalexin were time-dependent while the effects of lemongrass essential oil depended on both concentration and time. The main components of lemongrass essential oil identified by GC-MS were *trans*-citral (45.32% of total peak area) and *cis*-citral (35.43% of total peak area). The results of this study show that lemongrass essential oil had the potential to be used in combination with cephalexin for the control of superficial pyoderma in dogs caused by *S. pseudintermedius*.

**KEYWORDS**: antibiotic combination, cephalexin, lemongrass essential oil, *Staphylococcus pseudintermedius*,

## INTRODUCTION

*Staphylococcus pseudintermedius* is an important pathogen in veterinary medicine that causes infectious dermatitis in pets such as dogs and cats and can also be transmitted to humans [1]. This bacterium is resistant to many types of antibiotics, and treatment failure can result in chronic and latent infections in sick animals, leading to increased drug use [2]. Cephalexin is a first-generation cephalosporin, a subclass of beta-lactam antibiotics, which is widely used in veterinary medicine to treat diseases caused by Gram-positive and Gram-negative bacteria such as urinary tract infection, soft tissue infection, pneumonia, and pyoderma. It is an inexpensive, non-toxic, and broad-spectrum oral antibiotic that is resistant to staphylococcal beta-lactamase. However, as the repeat or prolonged use of antibiotics can induce drug resistance [3], there have been extensive studies into the antibacterial activity of novel natural substances such as essential oils, which are a mixture of many active substances with a broad spectrum of activity [4].

Studies into the use of essential oils in combination with antibiotics have shown that certain combinations of essential oils and antibiotics can promote the antimicrobial action of both [4]. Essential oil from oregano (*Origanum vulgare*) was synergistic with fluoroquinolones, doxycycline, lincomycin, and maquindox against *Escherichia coli*, rosemary oil (*Rosmarinus officinalis*) was synergistic with ciprofloxacin against *Klebsiella pneumoniae*, eucalyptus oil (*Eucalyptus obliqua*) was synergistic with chlorhexidine digluconate against *Staphylococcus epidermidis*, and lemongrass oil (*Cymbopogon citratus* (DC.) Stapf) was synergistic with kanamycin and streptomycin against *Salmonella* Typhimurium [5, 6]. Lemongrass is a freely available, inexpensive plant that is widely cultivated in tropical and subtropical regions of Asia, South America, and Africa [7]. Lemongrass essential oil has a wide range of pharmacological activities including anti-inflammatory, antioxidant, and antimicrobial effects [8]. Several reports have shown that lemongrass essential oil is highly effective against bacteria such as *Klebsiella pneumoniae*, *Staphylococcus au-*

*reus*, *Streptococcus agalactiae*, *Bacillus subtilis*, and *Bacillus cereus* [9–11]. However, there is limited information about synergism between lemongrass essential oil and cephalosporins. Therefore, we investigated the synergistic effects of lemongrass essential oil with cephalexin against *S. pseudintermedius* isolated from dogs with superficial pyoderma.

## MATERIALS AND METHODS

### Bacterial collection and identification

Bacterial samples were collected from skin lesions of dogs diagnosed with superficial pyoderma in the animal hospital of the Faculty of Veterinary Medicine, Khon Kaen University, Thailand. The bacteria were Gram-positive cocci arranged in grape-like clusters and produced pinpoint colonies that showed beta haemolysis on blood agar. Bacteria that were catalase and coagulase positive, oxidase and hyaluronidase negative, and produced acid from mannitol, sucrose, and trehalose were confirmed to be *S. pseudintermedius* by PCR-restriction fragment length polymorphism (PCR-RFLP) method as described previously [12]. Briefly, DNA was extracted by BacterialXpress™ nucleic acid extraction kit (Chemicon, Germany) and replicated using primers: pta_f1 (5′-AAA GAC AAA CTT TCA GGT AA-3′) and pta_r1 (5′-GCA TAA ACA AGC ATT GTA CCG-3′) (Bio Basic Canada Inc., Canada). The mixture was placed into a thermocycling machine (T100™ Thermocycler, Bio Rad, USA), and the conditions were set as follows: 95 °C for 2 min, then 35 cycles of 95 °C for 1 min, 53 °C for 1 min and 72 °C for 1 min, and finally 72 °C for 7 min. PCR products were digested with *Mbo*I (Promega, USA) for 4 h at 37 °C. The length of the pre-digested PCR product was 320 bp, and the digested products were 213 and 107 bp [13]. *S. pseudintermedius* isolates were preserved in Mueller-Hinton agar (MHA) at 4 °C until use. The *S. pseudintermedius* isolates were subcultured in Mueller-Hinton broth (MHB) (Both MHA and MHB were from Becton Dickinson, France) and incubated at 37 °C for 24 h before use, and $10^6$–$10^7$ CFU/ml bacterial concentrations were prepared by measuring the optical density at 600 nm ($OD_{600}$=0.03).

### Antibiotic susceptibility test by disk diffusion method

The inhibition zones against tested microorganisms were determined by the disk diffusion method according to CLSI [14]. Briefly, the microbial suspension ($10^6$–$10^7$ CFU/ml) was inoculated onto MHA plates by the streak plate technique. Antibiotic disks: 1 μg/disk oxacillin, 30 μg/disk cefoxitin, 10 μg/disk ampicillin, 10 μg/disk penicillin G, 30 μg/disk cefazolin, 15 μg/disk erythromycin, and 30 μg/disk chloramphenicol were placed onto the inoculated surface of each plate. The plates were incubated at 37 °C for 18–24 h. The inhibition zones were measured using a ruler. All tests were performed in triplicate. The zone diameters for each isolate were interpreted using the interpretive criteria of CLSI [15] (Table 1).

**Table 1** Zone diameter interpretive standards for *Staphylococcus* spp. [15].

| Antibiotic | Concentration (μg/disk) | Interpretive criteria (nearest whole mm) | | |
|---|---|---|---|---|
| | | S (⩾) | I | R (⩽) |
| Oxacillin (OX1) | 1 | 18 | – | 17 |
| Cefoxitin (FOX30) | 30 | 22 | – | 21 |
| Ampicillin (AMP10) | 10 | 29 | – | 28 |
| Penicillin G (P10) | 10 | 29 | – | 28 |
| Cefazolin (KZ30) | 30 | 23 | 20–22 | 19 |
| Erythromycin (E15) | 15 | 23 | 14–22 | 13 |
| Chloramphenicol (C30) | 30 | 18 | 13–17 | 12 |

A dash (–) indicates that interpretive criteria are not applicable. S = susceptible, I = intermediate, and R = resistant.

### Determination of lemongrass essential oil composition by GC-MS

The GC-MS analysis was performed according to the method previously described by Aiemsaard et al [9] with some modifications for the Agilent CN10402086 gas chromatograph and the Agilent US35120381 mass spectrometer (Agilent Technologies, USA). The column used was a DB-5ms fused silica capillary column (30 m × 25 mm, film thickness 0.25 μm). The carrier gas was helium with a flow rate of 1 ml/min. The oven temperature was increased from 70 to 120 °C at a rate of 3 °C/min, then from 120 to 270 °C at a rate of 5 °C/min. The chemical constituents of lemongrass essential oil were identified by comparing the mass spectrum of the sample with mass spectral libraries.

### Essential oil preparation

The essential oil of lemongrass (*Cymbopogon citratus*) was prepared by steam distillation and purchased from Thai-China Flavours and Fragrances Industry Co., Ltd, Thailand. A 100 mg/ml stock solution of each essential oil was prepared by dilution with a mixture of 5% (v/v) polyoxyethylene (20) sorbitan monooleate (Tween-80, Ajax Finechem Pty

Ltd., Australia) and 5% (v/v) ethyl alcohol (Merck, Germany).

### Determination of antibacterial activity of cephalexin and lemongrass essential oil by broth microdilution method

The MICs and MBCs of cephalexin and lemongrass essential oil were determined by broth microdilution method according to CLSI [14] with modifications. Briefly, 50 µl of MHB was added to all wells of a 96-well round-bottomed microtiter plate. The stock solution of each agent was added to each well of the first column, and serial twofold dilutions were performed from the first to the tenth column. Fifty microliters of bacterial suspension ($10^6$–$10^7$ CFU/ml) were added into all wells from the first to the eleventh columns. The wells of the eleventh and twelfth columns were used as positive (bacterial suspension and MHB) and negative (MHB only) growth controls, respectively. The plates were incubated at 37 °C for 24 h. A solution of 5% (v/v) Tween-80 and ethyl alcohol was used as solvent control. The MIC was determined from the lowest concentration of the antimicrobial agent inhibiting visible growth after 24 h of incubation. Ten microliters from each of the wells with no visible growth were inoculated onto MHA plates and incubated at 37 °C for 24 h. The MBC was determined from the lowest concentration of the antimicrobial agent that inhibited growth on MHA. All tests were performed in triplicate.

### Evaluation of synergistic effect of cephalexin and lemongrass essential oil by checkerboard method

The checkerboard method was performed according to the previous study of D'Arrigo et al [16] with modifications. Briefly, cephalexin was serially twofold diluted with MHB across the columns in 96-well round-bottomed microtiter plates to a final concentration range of 0.5–32 µg/ml. Fifty microliters of each concentration of lemongrass essential oil (twofold dilutions of 12.5 to 0.2 mg/ml) were added to each row. One hundred microliters of bacterial suspension ($10^6$–$10^7$ CFU/ml) were added into all tested wells. The wells containing only MHB and MHB with bacteria were used as negative and positive growth control wells, respectively. After 24 h at 37 °C, the MIC values of the cephalexin and lemongrass essential oil combination were determined. The fractional inhibitory concentration indices (FICI) were calculated using the following formula: FICI = ($MIC_{cephalexin}$ in combination/$MIC_{cephalexin}$ alone)+($MIC_{lemongrass\ essential\ oil}$ in combination/$MIC_{lemongrass\ essential\ oil}$ alone). FICI values less than or equal to 0.5 indicate synergistic effect, values greater than 0.5 but less than 1.0 indicate partial synergistic effect, values of 1.0 indicate an additive effect, values greater than 1.0 but less than 4.0 indicate indifferent effect, and values of 4.0 or greater indicate an antagonistic effect [17]. All tests were performed in triplicate.

**Table 2** Antibiotic susceptibility test of *S. pseudintermedius* isolates ($n = 7$).

| Antibiotic | Concentration (µg/disk) | Susceptible (%) | Resistant (%) |
|---|---|---|---|
| Oxacillin | 1 | 100 | 0 |
| Cefoxitin | 30 | 100 | 0 |
| Cefazolin | 30 | 100 | 0 |
| Chloramphenicol | 30 | 85.71 | 14.29 |
| Erythromycin | 15 | 71.43 | 28.57 |
| Ampicillin | 10 | 28.57 | 71.43 |
| Penicillin G | 10 | 28.57 | 71.43 |

### Time-kill study of cephalexin and lemongrass essential oil

The time-kill kinetics of cephalexin and lemongrass essential oil alone and combination were studied against *S. pseudintermedius* isolate number 2 according to Aiemsaard et al [18] with modifications. Briefly, each agent was mixed separately (alone) or together (combination) with 100 µl of bacterial suspension ($10^6$–$10^7$ CFU/ml) to give final concentrations of 1, 5, and 10 times their respective MICs in a total volume of 1000 µl. After incubation for 15 and 30 min, 3, 6, and 24 h at 37 °C, 100 µl of the mixture was 10-fold diluted with 0.89% sodium chloride solution to stop the antimicrobial activity of the agents. Then, 100 µl aliquots of the $10^{-1}$ to $10^{-3}$ dilutions were inoculated onto MHA plates. After incubation at 37 °C for 24 h, visible colonies of tested microorganisms were counted and recorded. Each experiment was performed in triplicate.

## RESULTS

### Clinical isolates of *S. pseudintermedius* and antibiotic susceptibility test

The bacterial isolates collected from skin lesions of dogs with superficial pyoderma were identified as *S. pseudintermedius* by biochemical tests and PCR-RFLP. The length of DNA fragment before digestion with *Mbo*I was 320 bp (Fig. 1A) while post-digested fragments were 213 and 107 bp (Fig. 1B). The results of disk diffusion antibiotic susceptibility

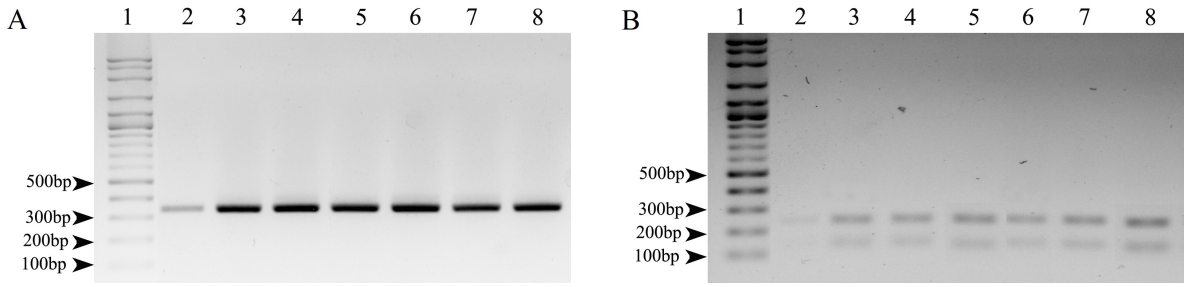


**Fig. 1** The agarose gel electrophoresis of *pta* PCR products. The products (A) before digested with *Mbo*I; (B) after digested with *Mbo*I. Lane 1 = 100-bp DNA ladder, lane 2 to 8 = *S. pseudintermedius* isolate number 1 to 7, respectively.

test showed that all tested bacteria were susceptible to oxacillin, cefoxitin, and cefazolin; 1 isolate (14.29%) was resistant to chloramphenicol, and 2 isolates (28.57%) were resistant to erythromycin. For ampicillin and penicillin G, only 2 isolates (28.57%) were susceptible (Table 2).

### Chemical composition of lemongrass essential oil

The results of GC-MS analysis identified 90.34% of the total lemongrass essential oil components. The tested essential oil contained 2 main and 4 minor constituents. The major components were *trans*-citral (geranial) and *cis*-citral (neral), accounting for 45.32 and 35.43% of total peak area, respectively. The minor components were *trans*-geraniol, 3,3,5-trimethylcyclohexene, 1-tert-butyl-3,3-dimethylcyclopropene, and geranyl acetate, which were in the concentration range of 1.45–4.12% of total peak area (Table 3).

### Antibacterial activity of cephalexin and lemongrass essential oil

The results of the broth microdilution tests of cephalexin and lemongrass essential oil against *S. pseudintermedius* isolates are shown in Table 4. There were only minimal differences in susceptibilities between isolates. Cephalexin MICs and MBCs for the 7 tested isolates were in the range of 1–4 and 2–16 μg/ml, respectively, with the $MIC_{90}$ value twice that of the $MIC_{50}$. The lemongrass essential oil MICs and MBCs for the 7 tested isolates were much higher than those of cephalexin (780–1560 and 1560 μg/ml, respectively).

### Antibacterial effect of cephalexin and lemongrass essential oil in combination

Table 5 shows the results of the antibacterial activity of cephalexin in combination with lemongrass essential oil. This combination showed partial synergy against all *S. pseudintermedius* isolates with the same FIC index value (0.75) for all 7 tested strains. Interestingly, the MICs of cephalexin and lemongrass oil in combination were 2–4 times less than those for cephalexin and lemongrass essential oil alone. This increased the antibacterial effect by 0.5–2 μg/ml of cephalexin and 390 μg/ml of lemongrass essential oil.

The results of the time-kill assays of cephalexin and lemongrass essential oil in combination and alone are given in Fig. 2. *S. pseudintermedius* isolate number 2 was used in this study since it was relatively more resistant to cephalexin than other isolates. The results demonstrated that the activity of lemongrass essential oil depended on both time and concentration, but the activity of cephalexin depended on time only. At a concentration of 1 time MIC, lemongrass essential oil alone (1560 μg/ml) reduced the number of viable bacteria from the initial inoculum ($1 \times 10^6$ CFU/ml) by about $1-\log_{10}$ at 6 h and by about $3-\log_{10}$ at 24 h, which was more than cephalexin alone (4 μg/ml) and the combination (1 μg/ml cephalexin combined with 390 μg/ml lemongrass essential oil) at 3, 6, and 24 h (Fig. 2A). When the concentration was increased to 5 times MIC, the combination of cephalexin (5 μg/ml) and lemongrass essential oil (1950 μg/ml) and lemongrass essential oil alone (7800 μg/ml) eradicated more than 90% ($1.0-\log_{10}$ reduction), 94% ($1.2-\log_{10}$ reduction), and 99.9999% ($6.0-\log_{10}$ reduction) of the viable bacteria at 3, 6, and 24 h, respectively, which was more effective than cephalexin alone (20 μg/ml) (Fig. 2B). The time-kill kinetics of antibacterial agents at 10 times MIC are shown in Fig. 2C. The results revealed that 40 μg/ml cephalexin had the same eradicating effect as 20 μg/ml cephalexin when used alone, reducing the number of viable bacteria by 0.6 and $2.37-\log_{10}$ at 6 and 24 h, respectively. In contrast, the combination (10 and

**Table 3** Chemical composition of lemongrass essential oil (*C. citratus*) as determined by GC-MS.

| Component | Molecular formula | Retention time (min) | Peak area (% of total peak area) |
|---|---|---|---|
| 1-tert-butyl-3,3-dimethylcyclopropene | $C_9H_{16}$ | 14.57 | 1.52 |
| 3,3,5-trimethylcyclohexene | $C_9H_{16}$ | 15.44 | 2.50 |
| Beta-citral, Neral, *cis*-citral, (Z)-citral | $C_{10}H_{16}O$ | 18.10 | 35.43 |
| *Trans*-geraniol | $C_{10}H_{18}O$ | 18.65 | 4.12 |
| Alpha-citral, geranial, *trans*-citral, (E)-citral | $C_{10}H_{16}O$ | 19.48 | 45.32 |
| Geranyl acetate | $C_{12}H_{20}O_2$ | 24.68 | 1.45 |

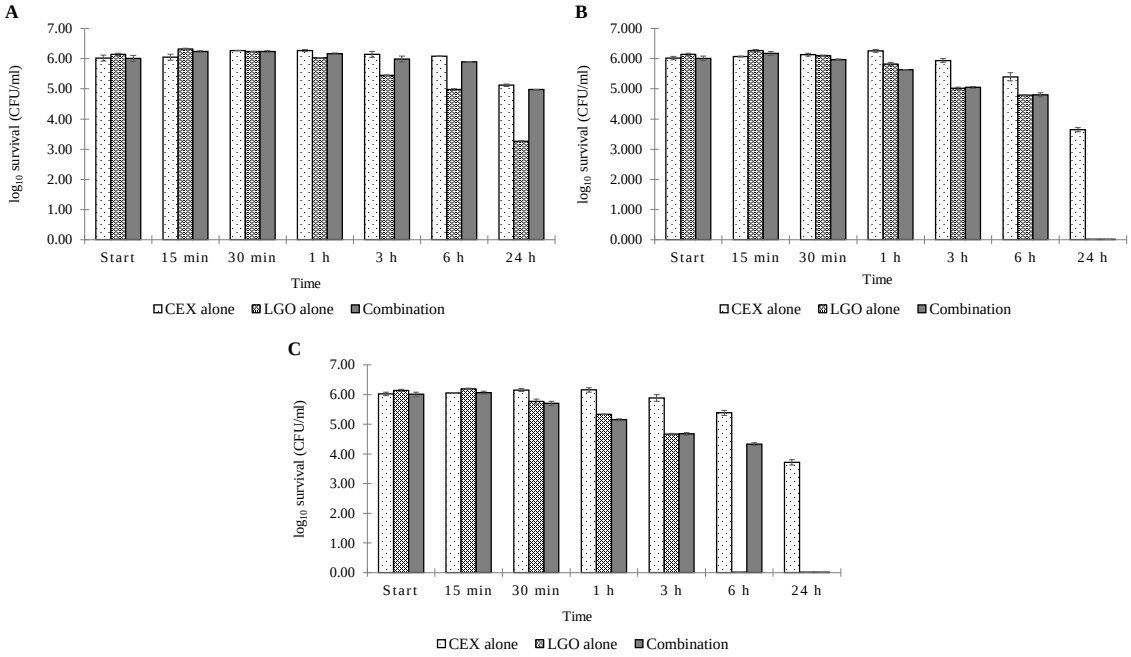


**Fig. 2** The time-kill assay of cephalexin (CEX) and lemon grass essential oil (LGO) against *S. pseudintermedius* isolate number 2. (A) 1 × MIC: alone (CEX 4 μg/ml or LGO 1560 μg/ml), combination (CEX 1 μg/ml + LGO 390 μg/ml); (B) 5 × MIC: alone (CEX 20 μg/ml or LGO 7800 μg/ml), combination (CEX 5 μg/ml + LGO 1950 μg/ml); (C) 10 × MIC: alone (CEX 40 μg/ml or LGO 15 600 μg/ml), combination (CEX 10 μg/ml + LGO 3900 μg/ml). Values represent means of triplicate with error bars (SD).

**Table 4** MICs and MBCs of cephalexin and lemongrass essential oil (LGO) against clinical isolates of *S. pseudintermedius* ($n = 7$).

| Agent | Antibacterial activity (μg/ml) | | | |
|---|---|---|---|---|
| | $MIC_{50}$ | $MIC_{90}$ | $MBC_{50}$ | $MBC_{90}$ |
| Cephalexin | 2 | 4 | 8 | 16 |
| LGO | 1560 | 1560 | 1560 | 1560 |

Values represent the MICs and MBCs collected from triplicate experiments.

3900 μg/ml of cephalexin and lemongrass essential oil, respectively) and lemongrass essential oil alone (15 600 μg/ml) showed an increased killing effect at 10 times MIC. At 30 min, 1, and 3 h, both the lemongrass essential oil alone and combination reduced the number of viable bacteria by 0.4, 0.9, and $1.4 - \log_{10}$, respectively. At later timepoints, the 10 times MIC lemongrass essential oil alone eradicated the bacteria after 6 h, and the 10 times MIC combination eradicated the bacteria after 24 h.

## DISCUSSION

Antibiotic susceptibility tests performed on the *S. pseudintermedius* isolates indicated that most tested isolates (5 of 7; 71.43%) were resistant to the aminopenicillins, ampicillin, and penicillin G. These resistant isolates are also likely to be resistant to other penicillinase-labile penicillins such as amoxicillin, carbenicillin, and ticarcillin [14]. This agrees with the previous study by Priyantha et al [19], who reported that 78% and 61% of *S. pseudinter-*

**Table 5** Synergistic effect of cephalexin and lemongrass essential oil (LGO) against *S. pseudintermedius* isolates.

| Isolate number | Agent | MIC (μg/ml) | | FIC (μg/ml) | FIC index[a] (μg/ml) | Outcome |
|---|---|---|---|---|---|---|
| | | Alone | Combination | | | |
| 1 | Cephalexin<br>LGO | 2<br>1560 | 1<br>390 | 0.50<br>0.25 | 0.75 | P |
| 2 | Cephalexin<br>LGO | 4<br>780 | 1<br>390 | 0.25<br>0.50 | 0.75 | P |
| 3 | Cephalexin<br>LGO | 2<br>1560 | 1<br>390 | 0.50<br>0.25 | 0.75 | P |
| 4 | Cephalexin<br>LGO | 2<br>1560 | 1<br>390 | 0.50<br>0.25 | 0.75 | P |
| 5 | Cephalexin<br>LGO | 1<br>1560 | 0.5<br>390 | 0.50<br>0.25 | 0.75 | P |
| 6 | Cephalexin<br>LGO | 4<br>1560 | 2<br>390 | 0.50<br>0.25 | 0.75 | P |
| 7 | Cephalexin<br>LGO | 2<br>1560 | 1<br>390 | 0.50<br>0.25 | 0.75 | P |

[a] FIC index was interpreted as synergy at $\leqslant 0.5$, partial synergy (P) at $> 0.5$ but $<1.0$, additive effect at 1.0, indifferent at $>1.0$ but $< 4.0$, and antagonistic when values were $\geqslant 4.0$.

*medius* isolated from dogs were resistant to penicillin and ampicillin, respectively. In contrast, all tested strains were sensitive to oxacillin, cefoxitin, and cefazolin. These penicillinase-resistant penicillins and cephalosporins are effective against penicillinase-producing *Staphylococcus* as they have different structures. For example, cephalosporins contain 7-aminocephalosporanic acid as a core structure, which provides beta-lactamase stability and good activity against penicillin-binding proteins [3]. However, the susceptibility or resistance to antibiotics for each bacterial strain depends on the expression of phenotypes and genotypes in bacterial cells [20].

Synergy occurs when the combined effect is better than the sum of individual effects. The checkerboard assay was used to test for the synergistic effect of cephalexin with lemongrass essential oil based on the comparison of MIC values determined for the substances alone and in combination. The results demonstrated that most isolates were susceptible to cephalexin alone, but some were interpreted as intermediate (susceptible: MIC $\leqslant$2 μg/ml, intermediate: MIC = 4 μg/ml, resistant: MIC $\geqslant$ 8 μg/ml [15]). The checkerboard assay indicated that lemongrass essential oil possessed a partial synergistic effect with cephalexin, and all tested isolates were susceptible to this agent. The time-kill assay showed that the combination of lemongrass essential oil with cephalexin had more eradicating activity than cephalexin alone. Like other beta-lactam antibiotics, cephalexin antibacterial activity is time-dependent. Cephalexin inhibits bacterial cell wall formation by inhibiting the activity of transpeptidase and peptidoglycan-active enzymes, also called penicillin-binding proteins, blocking cross-linking between glycopeptide polymer units [21]. Based on GC-MS analysis, steam distilled-lemongrass essential oil contains several constituents that have been shown to have antibacterial activity including citral (67.02–80.93%), geranial (37.58–45.95%), neral (29.44–34.98%), myrcene (5.64–15.69%), and geraniol (0.53–4.6%) [9, 22, 23]. Aiemsaard et al [24] previously showed that citral had antibacterial activity against *S. aureus* DMST4745 with MIC values between 0.62–1.25 μl/ml (554–1,116 μg/ml) while myrcene and geraniol had MIC values of 1.25 μl/ml and 0.54 μl/ml (approximately 482 μg/ml), respectively. These compounds affected the cell wall and the cell membrane of bacteria, causing morphological changes and leakage of intracellular substances. Also, citral decreased intracellular adenosine triphosphate (ATP) levels, which is an important source of energy for the metabolic and homeostatic processes of cells [25].

The synergism between antibiotics and essential oil may be attributed to the antibacterial activity of the chemicals in essential oil, which enhances the effect of antibiotics. The multiple antibacterial mechanisms of essential oil could make it harder for bacteria to develop resistance. Also, as the essential oil disrupts the cell wall and the cell membrane, this could allow the antibiotic to penetrate the bacterial cells more easily, thereby increasing its activity. In addition, decreasing the intracellular

ATP affects the processes of enzymatic reactions and signaling functions, resulting in the loss of dynamic equilibrium, an increase in intracellular pH, and the hyperpolarization of bacterial membrane potential, which is believed to help suppress the antibiotic resistance mechanism of bacteria [6]. Currently, there are limited studies on this topic, but there is one report that the combination of oregano essential oil with fluoroquinolones, doxycycline, lincomycin, maquindox, and florfenicol has the potential to inhibit extended-spectrum beta-lactamase production by *E. coli* [26]. Furthermore, Lorenzi et al [27] reported that essential oil of *Helichrysum italicum* can improve the activity of chloramphenicol against multi-drug resistant *Enterobacter aerogenes*, which is cross-drug class resistant due to the overproduction of protein efflux pumps.

In conclusion, lemongrass essential oil had activity against *S. pseudintermedius* isolated from dogs with superficial pyoderma. The FIC indices of the combination of cephalexin and lemongrass essential oil indicated partial synergy. Time-kill assays showed dose- and time-dependent killing by lemongrass essential oil alone and combination. The results of this study suggest that lemongrass essential oil can potentially be used in combination with cephalexin for controlling superficial pyoderma in dogs. Further studies in experimental animals are required to determine the appropriate concentrations, formulations, and treatment patterns for *in vivo* efficacy.

*Acknowledgements*: This work was supported by the Faculty of Veterinary Medicine, Khon Kaen University, Thailand. The authors thank Dr. Glenn Borlace, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand, for English language assistance.

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