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Full Length Research Paper

Pharmacodynamic properties of essential oils from *Cymbopogon* species

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Pharmacodynamic research plays an important role in the development of new antibacterial agents. Characterization of this pharmacodynamic can be used to design the best dose and dosing strategy for clinical trials. The pharmacodynamic properties can be determined by studying the bactericidal activity and the postantibiotic effects (PAE). Measurements of both bactericidal activity and the lag time could be useful in screening the efficacy of antimicrobial agents. In this study, the pharmacodynamic properties of essential oils from *Cymbopogon flexuosus* (lemongrass) and *Cymbopogon nardus* (citronella) as well as the combinations of both essential oils were evaluated against *Staphylococcus aureus* and *Escherichia coli.* At high concentrations (1.0 × minimum bactericidal concentration (MBC) and 0.5 × MBC), citronella and lemongrass essential oils alone or in combinations indicate high bactericidal activities toward *S. aureus* and *E. coli*, as shown by the decrease of optical absorbance values serially up to 24 h. However, these two essential oils or its combinations at lower concentrations (0.25 × MBC and 0.125 × MBC) showed the bacterial regrowth after 3 and 1 h of exposure time against *S. aureus* and *E. Coli*, respectively. Generally, citronella and lemongrass essential oils as well as its combinations indicate a significant lag of regrowth or PAE values which were more than 0.5 h towards both *E. coli* and *S. aureus*. This finding suggests that essential oils from *Cymbopogon* species showed a potential antimicrobial activity that can further be used for clinical treatment; thus, there is need for a study on the possible impact of PAE in the clinical situation.

Key words: *Cymbopogon* species, pharmacodynamic, postantibiotic, bactericidal activity, postantibiotic effects (PAE).

INTRODUCTION

Essential oils which are usually extracted from plant parts, such as leaves, fruits, flowers and seeds, have been of great use in food flavoring, fragrances, perfumes, aftershaves, aromatherapy and pharmaceuticals (Van de Braak and Leitjen, 1999). It has long been recognized that some essential oils have antimicrobial properties. For instance, Hammer et al. (1999) reported that essential oils derived from lemongrass had shown the antimicrobial activity against some pathogenic bacteria, such *as Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus*. Therefore, research on antimicrobial mechanism, such as pharmacodynamic study should be conducted.

Pharmacodynamic study plays an important role in the development of new antibacterial agents. The pharmacodynamic properties can be evaluated by studying the bactericidal activity and the postantibiotic effects (PAE). The pharmacodynamic data will help to define the clinical potentials of new drugs. It is also used to identify the strengths and weakness of these new drugs in comparison with synthetic drugs in the market. Hence, it is important in the development of new antibacterial or antimicrobial agents (Lister, 2006). PAE, a pharmacodynamic

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parameter that is contributed in choosing antibiotic doses, has to be determined after the antimicrobial activity of an antimicrobial agent or antibiotic is discovered (Pankuch and Appelbaum, 2006). PAE is defined as the length of time that bacterial growth is suppressed following brief exposure to an antibiotic (Pankuch and Appelbaum, 2006; Boswell et al., 1997). Thus, the aim of this present study is to determine the single and combine effects of essential oils from *Cymbopogon flexuosus* (lemongrass) and *Cymbopogon nardus* (citronella) on bactericidal activity and PAE towards *E. coli* and *S. aureus.*

MATERIALS AND METHODS

Preparation of bacterial inoculums

Two strains of bacteria used in this study, *E. coli* and *S. aureus* were obtained from microbiology laboratory, Faculty of Science and Technology (FST), Universiti Sains Islam Malaysia (USIM). Each bacterium was streaked onto Mueller-Hinton agar (MHA) and was incubated overnight at 37 ± 1°C. The isolated colony from each strain of bacteria was further inoculated in 10 ml of Mueller-Hinton broth (MHB) and was incubated at 37°C overnight. The densities of the bacterial inoculums were then measured using Biophotometer (Eppendorf AG, Hamburg, Germany) at 600 nm. The concentration of each bacterial inoculum was determined according to the McFarland standard formula. Each bacterial inoculum was then diluted to the concentration of 10^6 cfu/ml for further bactericidal activity and PAE studies.

Preparation of test samples

Essential oils of *C. nardus* (citronella) and *C. flexuosus* (lemongrass) used in this study were obtained from Universiti Putra Malaysia (UPM). The concentrations of both essential oils used were based on minimum bactericidal concentration (MBC), including 2 × MBC, 1 × MBC, 0.5 × MBC and 0.25 × MBC. Previous study by Rizal (2008) reported that treatment of lemongrass essential oil towards *E. coli* and *S. aureus* exhibited minimum bactericidal concentration (MBC) values of 1/40 and 1/20, respectively. Whilst, treatment of citronella essential oil towards both bacteria (*E. coli* and *S. aureus*) shows an intermediary bactericidal activity with MBC value of 1/10. Based on these MBC values, different concentrations of both essential oils were used for treatment with those two bacteria. In order to obtain the desired concentrations for this study, the dilution had been conducted by adding together certain amount of both essential oils and MHB. In this experiment, Streptomycin Sulfate was used as a positive control. In order to prepare 10 mg/ml of antibiotic solution, 10 mg of Streptomycin Sulfate (Sigma, St. Louis, USA) was added with 1 ml of sterilized distilled water.

Determination of Bactericidal Activity

The determination of bactericidal activity were done by using the method in Totsuka et al. (1999) and modified by Hanina (2006) by using the MBC instead of using MIC. Bacteria with the concentration of 10^6 plus the concentration of essential oil will be incubated at 37 °C in 0-24 h. After the interval of time desired, the addition of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, USA) was done to be next analyzed on the optical density of the mixture with microplate reader (BioTek ELx 800, US) at 630 nm. There were 3 controls for this experiment; one is for the time variables. 0 h will show that there is no growth of bacteria. For the treatment variable, one positive control and one negative control were made. The positive control was the addition of streptomycin, a type of antibiotic. The reason why the streptomycin were chosen was, it is one of the most susceptible and efficient antibiotic to the bacteria, as a result, no growth of bacteria. The other control, negative control was the inoculation of bacteria only without any antimicrobial agent. Since there is nothing to suppress the growth of the bacteria, the bacteria were grown.

Determination of postantibiotic effect (PAE)

Based on Boswell et al. (1997) and modified by Hanina (2006) from the preparation of bacterial inoculums, the bacteria were added with the essential oil. Then, it was incubated at 37 °C. The time to incubate the bacteria will be depending on the time for the bacteria to recover from the bactericidal activity experiment either 1 h or 3 h. Later, the bacteria were diluted with 1000 times of dilution using MHB to wash away the essential oil. Subsequently, incubation at 37°C with the time interval of 0 to 24 h follows. At the interval of time, the bacteria were plated on MHA. The agar plate were incubated overnight again at 37°C followed by the colony counting. For this experiment, the control was the treatment with streptomycin as the positive control. The negative control was the bacteria without any treatment.

RESULTS AND DISCUSSION

Postantibiotic effect is the term used to describe the continued suppression of the growth of an organism after a short exposure to an antimicrobial agent (Boswell et al., 1997). The PAE values were evaluated based on the bactericidal or killing effect of bacterial (*S. aureus* or *E. coli*) after short exposure to the mixtures of both essential oils. The incubation or exposure time for this treatment was dependent on the strains of bacteria used, which was related to the bactericidal activity. Based on bactericidal study, the exposure time for each *E. coli* and *S. aureus* was 1 and 3 h, respectively as shown in Figure 1.

PAE value was defined as the time required for one unit of logarithmic (1 $\log_{10}$) growth in the presence of drug minus the time required for that growth in the absence of drug or mathematically can be written as PAE = T - C, in which T is the time required for the test culture to increase 1 $\log_{10}$ from the original number of bacteria, while C refers to the time required for the control culture to increase 1 $\log_{10}$ from the original number of bacteria (Totsuka et al., 1999).

A significant lag of regrowth was defined as a lag of 0.5 h or more (Fuursted et al., 1997). Treatments of citronella and lemongrass essential oils alone and in combination at concentrations of 1 × MBC, 0.5 × MBC, 0.25 × MBC and 0.125 × MBC against *E. coli* showed PAE values more than 0.5 h, except for treatment with citronella oil that cannot be determined. Combination of citronella and lemongrass essential oils mixtures and single treatments at three concentrations of 1 × MBC, 0.5 × MBC and 0.25 × MBC towards *S. aureus* indicated that the PAE values more than 0.5 h. The PAE value for these two essential

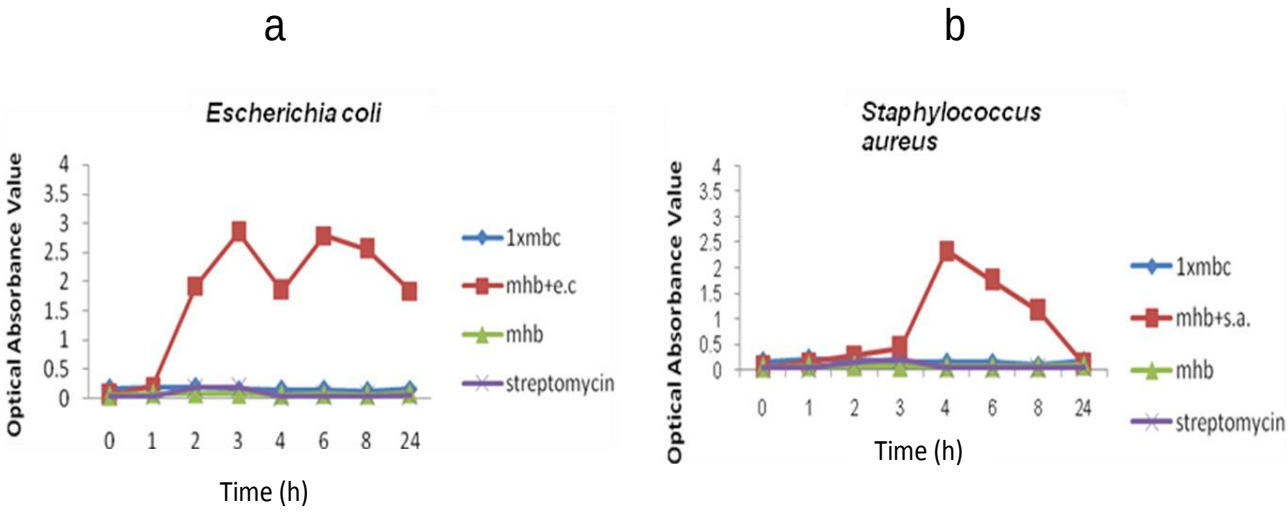

Figure 1. The time kill curve (optical absorbance against time) for the treatment of citronella and lemongrass essential oils against: (a) *E. coli* and (b) *S. aureus*.

Table 1. The PAE value of citronella and lemongrass essential oils at concentration of 1 × MBC, 0.5 × MBC, 0.25 × MBC and 0.125 × MBC.

Sample	PAE values (h)			
	1 × MBC	0.5 × MBC	0.25 × MBC	0.125 × MBC
EC + CF	6	2	2	1
EC + CN	6	2	0.5	0
EC + CM	4	4	3	3
SA + CF	6	3	3	0.5
SA + CN	4	3	1	0
SA + CM	2	2	1	0

CF - *Cymbopogon nardus*, CN - *Cymbopogon flexuosus*, CM - Combination of CN and CF, EC - *Escherichia.coli* and SA - *Streptococcus aureus.*

oils alone or in combination at lower concentration of 0.125 × MBC against *S. aureus* that cannot be determined showed no lag phase since the bacteria grew rapidly.

Based on Boswell et al. (1997), the antimicrobial agents that possessed longer PAE value than the exposure time are more effective in antimicrobial therapy when treating moderately susceptible organisms. The result of PAE values are shown in Table 1. In this study, treatment of citronella and lemongrass essential oils in combination with high (1 × MBC and 0.5 × MBC) and low concentrations (0.25 × MBC and 0.125 × MBC) against *E. coli*, showed longer inhibition time (3 to 4 h) than the exposure time (1 h). As a result, these combinations of essential oils with various concentrations might be used for killing moderately susceptible organisms.

Treatments of essential oils mixtures with both high concentrations and lower concentrations against *E. coli* showed PAE values of 4 and 3 h, respectively. Trounce and Gould (1994) reported that antibiotic is given in 3 to 6 h intervals per dose every day depending on the type of antibiotic and bacterial infections to retain the bactericidal level in the blood. Thus, combination of citronella and lemongrass essential oils might further be used for clinical treatment toward *E. coli* since these mixtures could delay the bacterial growth or maintain the bactericidal level within 3 to 4 h.

Citronella and lemongrass essential oils mixtures at concentration of sub-lethal dose (0.5 × MBC) showed PAE values of 4 and 2 h toward *E. coli* and *S. aureus,* respectively. Fuursted et al. (1997) reported that PAE effect in sub-lethal dose (sub-MBC) or sub-minimal inhibitory (sub-MIC) is a useful parameter in the formation of disinfectant. Therefore, these essential oils mixtures at sub-lethal concentrations might further be used as disinfectant.

Unlike in the single treatment, the highest PAE value (6h) was indicated by the treatment of lemongrass essential oil at concentration of 1 × MBC towards both *S. aureus* and *E. coli*. This was followed by 0.5 × MBC and

0.25 × MBC of lemongrass essential oil (with PAE value of 3 h) that was used in the treatment of *S. aureus*. Lemongrass essential oil at concentrations of 0.5 × MBC and 0.25 × MBC also showed a significant PAE value (2 h) against *E. coli*. At the lowest concentration (0.125 × MBC), lemongrass essential oil showed PAE values of 0.5 and 1 h for the treatment of *S. aureus* and *E. coli*, respectively.

The lag of regrowth clearly depended on the type of bacterial species and concentration of antibacterial agent used. Generally, lemongrass essential oil works effectively towards both bacterial species as the PAE values of this essential oil at each concentration ranging from 0.5 to 6 h. Lemongrass essential oil showed a good efficacy in treating both bacteria (*S. aureus* and *E. coli*).

In the PAE experiment with citronella essential oil, three concentrations (1 × MBC, 0.5 × MBC and 0.25 × MBC) showed significant lag of regrowth or PAE values against both tested bacteria which were *S. aureus* and *E. coli*. The PAE value of citronella essential oil with lower concentration of 0.125 × MBC for both *S. aureus* and *E. coli* that cannot be determined showed that the lag of regrowth or PAE value was not significant. The best PAE value (6 h) was indicated by the treatment of citronella essential oil at concentration of 1 × MBC against *E. coli*. This was followed by 1 × MBC of citronella essential oil (with PAE value of 4 h) that was used for the treatment of *S. aureus*.

In this postantibiotic effect study, essential oil extracted from *C. nardus* (L.) Rendle seems to have little impact on *S. aureus as* compared to *E. coli*. This is due to the composition of *S. aureus* in which this strain of bacteria had the ability to form biofilms. The biofilms are complex structures consisting of surface-attached bacteria surrounded by a self-reproduced extracellular polymer matrix. Bacteria with this properties exhibit elevated resistance to both antibiotics and the host-defence systems, and finally results in persistent and difficult-to-treat infections (Kwiecinski et al., 2009). Moreover, the citronella oil is not strong enough and not suitable in the treatment of dangerous bacteria like *S. aureus* as the thickened cell walls with multiple layers were observed after exposing *S. aureus* to protein synthesis inhibitory antibiotics, because of the changes in morphologies (Watanabe et al., 1997).

The importance of the PAE study lies in the fact that the antibiotic may be effective as a continuous dosage with an associated reduction in costs and toxicity, as well as a reduced risk for selection of resistance due to poor patient compliance, which results to suboptimal dosing regimens by allowing long PAEs for fewer daily doses (Geli et al., 2008). However, different PAE studies with the same organisms and antibiotics have come to wide range of conclusions which increases the difficulty of using PAE in the design of optimal dosing regimens (Geli et al., 2008).

Conclusions

Conclusively, citronella and lemongrass essential oils as well as its combinations indicate a significant lag of regrowth or PAE values which were more than 0.5 h towards both *E. coli* and *S. aureus*. This finding suggests that essential oils from *Cymbopogon* sp. showed a potential antimicrobial activity that can further be used for clinical treatment; thus, there is need for a study on the possible impact of PAE in the clinical situation.

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