

Pharmacodynamic properties of essential oils from cymbopogon biology essay

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Pharmacodynamic research plays an important function in the development of new antibacterial agents. A word picture of this pharmacodynamic can be used to plan the best dosage and dosing scheme for clinical tests.

The pharmacodynamic belongings can be determined by analyzing the bactericidal activity and the postantibiotic effects (PAE). Measurements of both disinfectant activity and the slowdown clip could be utile in testing the efficaciousness of antimicrobial agents. In this survey, the pharmacodynamic belongings of indispensable oils from *Cymbopogon flexuosus* (lemon grass) and *Cymbopogon nardus* (citronella) every bit good as the combinations of both indispensable oils were evaluated against *Staphylococcus aureus* and *Escherichia coli*. At high concentrations (1.

0xMBC and 0. 5xMBC) , citronella and lemon grass indispensable oils entirely or in combinations indicated high bactericidal activities toward *S. aureus* and *E.*

coli, as shown by the lessening of optical density values serially up to 24 hours. However, these two indispensable oils or its combinations at lower concentrations (0. 25xMBC and 0. 125xMBC) showed the bacterial regrowth after 3 hours and 1 hr of exposure clip against *S. aureus* and *E. coli* severally. Generally, citronella and lemon grass indispensable oils every bit good as its combinations indicated a important slowdown of regrowth or PAE values which were more than 0. 5 H towards both *E.*

coli and *S. aureus*. This determination suggests that indispensable oils from *Cymbopogon* sp.

showed a possible antimicrobial activity that can be further used for clinical intervention and demand warrant survey on the possible impact of PAE in the clinical state of affairs. Keywords: Pharmacodynamic ; Postantibiotic ; Cymbopogon sp. ; Bactericidal activity ; PAE.

Introduction

Essential oils which are normally extracted from various parts such as foliage, fruits, flowers and seeds, have been great usage in nutrient flavorer, aromas, aftershaves, aromatherapy and pharmaceuticals (Van de Braak & A ; Leitjen, 1999) . It has long been recognized that some indispensable oils have antimicrobial properties. For case, Hammer et Al. (1999) reported that indispensable oils derived from lemon grass had shown the antimicrobial activity against some infective bacteriums such as Escherichia coli, Salmonella typhimurium and Staphylococcus aureus. Therefore, research on mechanism of antimicrobial action such as pharmacodynamic survey should be conducted.

Pharmacodynamic survey plays an of import function in the development of new antibacterial agents. The pharmacodynamic properties can be evaluated by analyzing the bactericidal activity and the postantibiotic effects (PAE) . The information will assist to specify the clinical potencies of new drugs. It is besides used to place the strengths and failing of these new drugs as a comparing with man-made drugs in the market. Hence, it is of import in the development of new antibacterial or antimicrobial agents (Lister, 2006) . Postantibiotic consequence (PAE) or pharmacodynamic parametric quantity that is contributed in taking antibiotic doses has to be

determined after the antimicrobial activity of an antimicrobial agent or antibiotic is discovered (Pankuch et al.

, 2003) . PAE is defined as the length of time that bacterial growing is suppressed following brief exposure to an antibiotic (Pankuch et al. , 2003 ; Boswell et al.

, 1997) . Therefore, the purpose of this present survey is to find the individual and combination effects of indispensable oils from *Cymbopogon flexuosus* (lemon grass) and *Cymbopogon nardus* (citronella) on bactericidal activity and PAE towards *Escherichia coli* and *Staphylococcus aureus*.

MATERIALS AND METHODS

2.

1 Preparation of Bacterial Inoculums

Two strains of bacteria used in this survey, *Escherichia coli* and *Staphylococcus aureus* were obtained from microbiology research lab, Faculty of Science and Technology (FST) , Universiti Sains Islam Malaysia (USIM) . Each bacteria was streaked onto Mueller-Hinton agar (MHA) and incubated overnight at 37°C. The stray settlement from each strain of bacterium was further inoculated in 10ml of Mueller-Hinton stock (MHB) and incubated at 37°C overnight. The denseness of the bacterial inoculants were so measured utilizing Biophotometer (Eppendorf AG, Hamburg, Germany) at 600 nanometer. The concentration of each bacterial inoculant was determined harmonizing to the McFarland Standard expression. Each

bacterial inoculant was so diluted to the concentration of 10^6 cfu/ml for further bactericidal activity and postantibiotic consequence (PAE) surveys.

2. 2 Preparation of Test Samples

Cymbopogonardus (citronella) indispensable oil and Cymbopogonflexuosus (lemon grass) indispensable oil used in this survey were obtained from Universiti Putra Malaysia (UPM) . The concentrations of both indispensable oils used in this survey were based on minimal disinfectant concentration (MBC) , including $2 \times \text{MBC}$, $1 \times \text{MBC}$, $0.5 \times \text{MBC}$ and $0.25 \times \text{MBC}$. Previous survey by Rizal (2008) reported that intervention of lemon grass indispensable oil towards E. coli and S. aureus exhibited MBC values of $1/40$ and $1/20$ severally.

Whilst, intervention of citronella indispensable oil towards both bacteriums (E. coli and S. aureus) shown an intermediary bactericidal activity with MBC value of $1/10$. Based on these MBC values, different concentrations of both indispensable oils were used for intervention with those two bacteriums. In order to obtain the coveted concentrations for this survey, the dilution had been conducted by adding together certain sum certain sum of both indispensable oils and MHB. In this experiment, Streptomycin Sulfate was used as a positive control.

In order to fix 10 mg/ml of antibiotic solution, 10 milligram of Streptomycin Sulfate (Sigma, St. Louis, USA) was added with 1 milliliters of sterilized distilled H₂O.

2. 3 Determination of Bactericidal Activity

The finding of disinfectant activity were done by utilizing the method in Totsuka et Al. (1999) and modified by Hanina (2006) . Bacteria with the concentration of 10^6 plus the concentration of indispensable oil will be incubated at 37°C in 0-24 hours.

After the interval of clip desired, the add-on of MTT was done to be following analyzed on the optical denseness of the mixture with Elisa reader at 630nm. There were 3 controls for this experiment ; one is for the clip variables. 0 hr will demo that there is no growing of bacteriums. For the intervention variable, one positive control and one negative control were made. The positive control was the add-on of streptomycin, a type of antibiotic. The ground why we are taking the streptomycin since it is one of the most susceptible and efficient antibiotic to the bacteriums, as a consequence, no growing of bacteriums. The other control, negative control was the vaccination of bacteriums merely without any antimicrobial agent. Since there is nil to stamp down the growing of the bacteriums, the bacteriums were grown.

2. 4 Determination of Postantibiotic consequence (PAE)

Based on Boswell et Al. (1997) and modified by Hanina (2006) from the readying of bacterial inoculants, the bacteriums were added with the indispensable oil.

Then it was incubated with at 37°C . The clip to incubate the bacteriums will be depending on the clip the bacteriums recover from the bactericidal activity experiment either 1h or 3h. Subsequently, the bacteriums were

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diluted with 1000 times of dilution utilizing MHB to rinse away the indispensable oil. Subsequently follow with incubation at 37°C with the interval of clip 0-24 hours. At the interval of clip, the bacteriums were plated on MHA.

The agar home base were incubated overnight once more at 37°C followed by the settlement numeration. For this experiment, the control was the intervention with streptomycin as the positive control. The negative control was the bacteriums without any intervention.

RESULTS AND DISCUSSION

Postantibiotic consequence is the term used to depict the continued suppression of the growing of an being after a short exposure to an antimicrobial agent (Boswell et al. , 1997) . The PAE values were evaluated based on the bactericidal or killing consequence of bacterial (*S. aureus* or *E. coli*) after short exposure to the mixtures of both indispensable oils. The incubation or exposure clip for this intervention was dependent on the strains of bacteriums used, which was related to the bactericidal activity. Based on bactericidal survey, the exposure clip for each *E. coli* and *S.*

aureus was 1 H and 3 H severally as shown in Figure 1. a) B)Figure 1: The clip kill curve (optical optical density against clip) for the intervention of citronella and lemon grass indispensable oils against a) *Escherichia coli* and B) *Staphylococcus aureus*. PAE value was defined as the clip required for one unit of logarithmic (1 log₁₀) growing in the presence of drug minus the clip required for that growing in the absence of drug or mathematically can be written as $PAE = T - C$, in which T is the clip required for the trial civilization

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to increase 1 log₁₀ from the original figure of bacteriums, while C refer to the clip required for the control civilization to increase 1 log₁₀ from the original figure of bacteriums (Totsuka et al.

, 1999) . A important slowdown of regrowth was defined as a slowdown of 0.5 H or more (Fuursted et al, . 1997) . Treatments of citronella and lemon grass indispensable oils entirely and in combination at concentrations of 1xMBC, 0.5xMBC, 0.25xMBC and 0.125xMBC against E.

coli showed PAE values more than 0.5 H except for the intervention with citronella oil can non be determined. Combination of citronella and lemon grass indispensable oils mixtures and individual interventions at three concentrations of 1xMBC, 0.5xMBC and 0.25xMBC towards S.

aureus indicated that the PAE values more than 0.5 h. The PAE value for these two indispensable oils entirely or in combination at lower concentration (0.125xMBC) against S. aureus can non be determined, showed no slowdown stage since the bacterium grew quickly. Table 1: The PAE value of citronella and lemon grass indispensable oils at concentration of 1xMBC, 0.5xMBC, 0.

25xMBC and 0.125xMBC.

Samples

A

PAE Values (H)

1xMBC 0.5xMBC 0.

25xMBC0. 125xMBC

EC + CF

6221

EC + CN

620. 50

EC + CM

4433

SA + CF

6330. 5

SA + CN

4310

SA + CM

2210CF - *Cymbopogon nardus*, CN - *Cymbopogon flexuosus*, CM -

Combination of CN and CF, EC - *Escherichia. coli* and SA - *Streptococcus aureus* 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 handling reasonably susceptible beings. The consequence of PAE values are shown in Table 1. In this survey, intervention of citronella and lemon grass indispensable oils in combination with high (1xMBC and 0. 5xMBC) and low concentrations (0. 25xMBC and 0.

125xMBC) against *E. coli*, showed longer suppression clip (3 H to 4 H) than the exposure clip (1 H) . As a consequence, these combinations of indispensable oils with assorted concentrations might be used for killing reasonably susceptible beings. Treatments of indispensable oils mixtures with both high concentrations and lower concentrations against *E. coli* showed PAE values of 4 H and 3 H, severally. Study by Trounce & A ; Gould (1994) reported that antibiotic is given in 3-6 H intervals per dose every twenty-four hours depending on the type of antibiotic and bacterial infections to retain the bactericidal degree in the blood. Therefore, combination of citronella and lemon grass indispensable oils might be further used for clinical intervention toward *E.*

coli since these mixtures could detain the bacterial growing or keep the disinfectant degree within 3 H to 4 H. Citronella and lemon grass indispensable oils mixtures at concentration of sub-lethal dosage (0.5xMBC) showed PAE values of 4 H and 2 H toward *E.*

coli and *S. aureus* severally. Fuursted et Al. (1997) reported that PAE consequence in sub-lethal dosage (sub-MBC) or sub-minimal inhibitory (sub-MIC) is a utile parametric quantity in the formation of germicide. Therefore, these indispensable oils mixtures at sub-lethal concentrations might be farther used as germicide. Unlike in the individual intervention, the highest PAE value (6 H) was indicated by the intervention of lemon grass indispensable oil at concentration of 1xMBC towards both *S.*

aureus and *E. coli*. This was followed by 0.5xMBC and 0.

25xMBC of lemon grass indispensable oil (with PAE value of 3 H) that were used in the intervention of *S. aureus*. Lemongrass indispensable oil at concentrations of 0. 5xMBC and 0.

25xMBC besides showed a important PAE value (2 H) against *E. coli*. At the lowest concentration (0. 125xMBC) , lemongrass indispensable oil showed PAE values of 0.

5 H and 1 H for the intervention of *S. aureus* and *E. coli* severally. The slowdown of regrowth clearly depended on type of bacterial species and concentration of antibacterial agent used. By and large, lemongrass indispensable oil works efficaciously towards both bacterial species as the PAE values of this indispensable oil at each concentration runing from 0. 5 H to 6 h.

Lemongrass indispensable oil showed a good efficaciousness in handling both bacteriums *S. aureus* and *E. coli*. In the PAE experiment with citronella indispensable oil, three concentrations (1xMBC, 0. 5xMBC and 0. 25xMBC) showed important slowdown of regrowth or PAE values against both tested bacteriums which were *S. aureus* and *E.*

coli. The PAE value of citronella indispensable oil with lower concentration (0. 125xMBC) toward both *S.*

aureus and *E. coli* can non be determined, showed that the slowdown of regrowth or PAE value was non important. The best PAE value (6 H) was indicated by the intervention of citronella indispensable oil at concentration of 1xMBC against *E. coli*. This was followed by 1xMBC of citronella

indispensable oil (with PAE value of 4 H) that was used for the intervention of *S. aureus*. In this postantibiotic consequence survey, indispensable oil extracted from *Cymbopogon nardus* (L.) Rendle seems to hold small impact on *Staphylococcus aureus* compared to *Escherichia coli*.

This is due to the composing of *S. aureus* in which this strain of bacteriums had the ability to organize biofilms. The biofilms are complex constructions dwelling of surface-attached bacteriums surrounded by a self-reproduced extracellular polymer matrix. Bacteria with this belongings exhibit elevated opposition to both antibiotics and the host-defence systems and eventually consequences in relentless and difficult-to-treat infections (Kwiecinski et al. , 2009) . Furthermore, the citronella oil is non strong plenty and non suited in the intervention of unsafe bacteriums like *S.*

aureus as the thickened cell walls with multiple beds were observed after exposing *S. aureus* to protein synthesis inhibitory antibiotics because of the alterations in morphologies (Watanabe et al. , 1997) . In decision, the importance of PAE survey lies in the fact that how does the antibiotic may be effectual as uninterrupted dosing with an associated decrease in costs and toxicity every bit good as reduced hazard for choice of opposition due to hapless patient conformity and ensuing suboptimal dosing regimens by letting long PAEs for fewer day-to-day doses (Dominguez et al. , 2001 ; Geli et al.

, 2008) . However, different PAE surveies with the same beings and antibiotics have come to broad scope of decisions which increases the

trouble of utilizing PAE in the design of optimum dosing regimens (Geli et al. , 2008) .