INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

THE ANTIBACTERIAL ACTIVITIES OF AROMATHERAPY ESSENTIAL OILS OF LAVENDER (*LAVANDULA ANGUSTIFOLIA* MILL), *ROSEMARY* (*ROSMARINUS OFFICINALIS* L.) AND YLANG-YLANG (*CANANGA ODORATA* (LAMK.) HOOK) AGAINST AIRBORNE BACTERIA

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Article Received on: 07/06/18 Approved for publication: 26/06/18

DOI: 10.7897/2230-8407.09692

ABSTRACT

Essential oils have long been used in history to help aid a variety of health concerns. With the wide range of aromatherapy oils available, people are finding more and more uses for them, especially in the home and at work in getting good air quality. Many ways are made to achieve good air quality and free from microorganisms both pathogens and non-pathogens with the use of air filters, ultraviolet light, and which is now developed with aromatherapy. Lavender (*Lavandula angustifolia* Mill), rosemary (*Rosmarinus officinalis* L.), and ylang-ylang (*Cananga odorata* (Lamk.) Hook) essential oils have been used as aromatheraphy. The observing ungrowth bacterial colonies based on the time of evaporation of aromatherapy and the concentration of essential oils, can be searched that has the best antibacterial effect. The replica platting method was used in this research to conduct antibacterial activities, determined the inhibitory concentration and to determine the evaporation time of lavender, rosemary, and ylang-ylang essential oils. The concentration variation of essential oil was 5%, 7.5%, and 10% v/v and also variation of evaporation time during 30, 60, and 90 min. The result showed that a minimum inhibitory concentration respectively, 5% v/v with effective duration in 60 min for lavender and ylang-ylang essential oils that be required to have the effect on reducing the population of airborne bacteria and 7.5% for rosemary with effective duration in 90 min.

Keyword: Airborne, Antibacterial, aromatheraphy, Lavender, Rosemary, Ylang-ylang.

INTRODUCTION

Form of alternative medicine that uses volatile plant materials, known as essential oils and other aromatic compounds for the aromatherapy is a purpose of altering a person's mind, mood, cognitive function or health. Some essential oils such as tea tree have demonstrated anti-microbial effects, but there is still a lack of clinical evidence demonstrating efficacy against bacterial, fungal, or viral infections. Evidence for the efficacy of aromatherapy in treating medical conditions remains poor, with a particular lack of studies employing rigorous methodology, but some evidence exists that essential oils may have therapeutic potential[1].

Essential oil can be inhaled, massaged onto the skin, diffused into the air, applied as a compress, or placed in a bath for soaking. Diffusion is normally used to calm or soothe nerves or treats some respiratory problems and can be done by spraying oil-containing compounds into the air in fashion similar to an air freshener. It can also be done by placing a few drops of essential oil in a diffuser and turning on the heat source. Sitting within threefeet of the diffuser, a treatment normally lasts about 30 min[2,3].

Aromatherapy is a method of healing by using the aroma power derived from essential oil. In developed countries like England, essential oil has been used as an antiseptic to kill germs that are transmitted through the air in public places. The use of chemical antiseptic as airborne bacteria killers has side effects because it can leave residues or cause microbial resistance. Therefore, using of aromatherapy oil can kill microorganisms or reduce the number of airborne bacteria because in general essential oil contain chemical compounds such as alcohols, phenols, esters, sesquiterpenes that have antiviral, antifungal, bacteriostatic or bactericidal effects[4,5]. There are several types of aromatherapy that have antibacterial effects, such as tea tree oil, rose, sandalwood, lavender, geranium, ylang-ylang, eucalyptus, peppermint, rosemary, thyme, and others[6-8].

Research on the antibacterial effects of lavender, rosemary, and ylang-ylang oils is necessary to maximize the potential of this plant. Testing was done to reduce the number of bacteria in a room so we can get the clean and healthy air quality. The test was conducted by replica platting with observing ungrowth bacterial colonies based on the evaporation time of aromatherapy and the concentration of lavender, rosemary and ylang-ylang essential oils, so the best antibacterial effect can be searched. Evaporation of this essential oils using electric aromatherapy equipment (diffuser).

MATERIALS AND METHODS

Material

The material used in this research were lavender 100% purity essential oil, rosemary 100% purity (Narwastu®) and ylang-ylang 100% purity essential oils (Bali Tangi®). Alcohol 70% (Brataco®), aquadest (Brataco®), trypticase soy agar (TSA) (E-Merck®).

Instruments/equipment/apparatus

Aromatherapy diffuser (Kris®), Autoclave (All American™), spirit lamp (Pyrex®), Petri dish (Pyrex®), incubator

(Memmert™), test tube rack (standard), test tubes (Pyrex®), digital balance (Ohaus™), laminar air flow cabinet (Esco™).

Test Room

Used spaces that have a volume of ± 16 m^3. The study was conducted when the room was unused and closed, containing 4 small wooden chairs and one laminar air flow cabinet. Aromatherapy diffuser in the center of the room was placed on a chair.

Tools preparation

The tools to be used in the research should be washed, dried, and wrapped in paper. Then sterilized beforehand so as not to be contaminated using autoclave for 30 min with pressure of 15 dyne/cm^3 (1 atm) and temperature 121ºC.

Preparation of Trypton Soy Agar (TSA)

TSA making was done by weighing 40 g of TSA dissolved with aquadest 1 l and heated until dissolved, then sterilized in autoclave. Then a TSA media solution with a temperature of <45ºC was put into a sterile Petri dish as much as ± 20 ml aseptically.

Preparations of Lavender, Rosemary, and Ylang-ylang Essential Oils

The concentration of essential oil of lavender, rosemary, and ylang-ylang used was 5% (4 ml essential oil plus 76 ml aquadest), 7.5% (6 ml essential oil plus aquadest 74 ml), and 10% (essential oil 8 ml plus aquadest 72 ml).

Test Procedure

The first step was collected bacterial sampling with five Petri dishes containing TSA media placed at five points of equal distance over small wooden chairs and left open in the test room for 30 min under closed room conditions and at room temperature. After 30 min, the five petri dishes were closed and incubated at 35-37°C for 18-24 h. After incubation, choose 10 colony of bacteria from five petri dishes then cultured in inclined media and incubated at 35-37°C for 18-24 h. The ten bacteria were bred again into a Petri dish containing TSA media that has been divided into 10 boxes and used as a master plate.

Fabric that used to taste the master plate stamped first using the stamp tool that provided into the cup containing the TSA media as a fabric control. Then the master plate was stamped into two dishes containing new TSA media, test plate and secondary. Both processes were carried out in a laminar air flow cabinet. The dish I was placed in an open space in the test room which had previously evaporated the room with lavender, rosemary and ylang ylang essential oils each for 30, 60, and 90 min. Then the dish I was closed, while the secondary plate was immediately closed and incubated at 35-37°C for 18-24 h. Evaporation of essential oils of lavender, rosemary, and ylang-ylang were done with variation of concentration 5%, 7,5%, and 10% and each treatment was done three times repetition[9].

Observation of Bacterial Colonies

After incubation, observed ungrowth bacterial colonies in each TSA petri dish after evaporation with essential oils of lavender, rosemary and ylang-ylang.

Gram staining

The smear on a glass slide was covered with few drops of one of the primary stains. Gentian violet was a mixture of methyl violet and crystal violet. The primary stain renders all the bacteria uniformly violet. After a minute of exposure to the staining solution, the slide was washed in water. The smear was treated with few drop of Gram's Iodine and allowed to act for a minute. This results in formation of a dye-iodine complex in the cytoplasm. Gram's iodine serves as a mordant. The slide was again washed in water and then decolorized in absolute ethyl alcohol or acetone. A mixture of ecetone-ethyl alcohol (1:1) can also be used for decolorization. The process of decolorization was fairly quick and should not exceed 30 sec for thin smears. Acetone was a potent decolorizer and when used alone can decolorize the smear in 2-3 sec. A mixture of ethanol and acetone acts more slowly than pure acetone. Decolorization was the most crucial part of Gram staining and errors can occur here. Prolonged decolorization can lead to over-decolorized smear and a very short decolorization period may lead to under-decolorized smear. After the smear was decolorized, it was washed in water without any delay.

The smear was finally treated with few drops of counterstain such as dilute carbol fuchsin, neutral red or safranin. The slide was washed in water; excess water was removed using a blotting paper, dried in air and heat fixed before observing under microscope. Those bacteria that hold on to primary dye-iodine complex and remain violet were called Gram positive and those which get decolorized and subsequently take up counterstain (pink/red) were called Gram negative[10].

RESULTS AND DISCUSSION

The aromatherapy essential oils of lavender, rosemary, and ylang-ylang had antibacterial activities. Viewed from the presence of bacterial colonies that ungrowth after being evaporated with the essential oil using aromatherapy diffuser distinguished by concentration variations, ie 5%, 7.5%, and 10% and variations of time for 30, 60, and 90 min and each time variation was repeated three times. The results can be seen in Table 1 and Figure 1.

The result showed that lavender aromatherapy has an effect on concentrations of 5% and 7.5% with effective evaporation time of up to 60 min, while at 10% having an effective time of up to 90 min.

The result showed that rosemary aromatherapy at a concentration of 5% at each time interval has no effect at all, and only gives effect at 7.5% with effective time after 90 min and for 10% has effective time only until 30 min.

At a concentration of 10% in the 30th min, the rosemary aromatherapy had an average percentage of bacterial colonies that ungrowth by 20% and at a concentration of 7.5% in the 90th min had an average percentage of 10%, can be seen in Figure 2.

The result showed that ylang-ylang aromatherapy has effect at 5% concentration with effective time up to 60 min, while at concentration of 7.5% and 10% has effective time up to 90 min. At concentrations of 5% and 7.5% in the 30th and 60th min, and 10% concentration in the 90th min aromatherapy of ylang-ylang essential oil had an average percentage of 10% ungrowth bacterial colonies. At a concentration of 7.5% in the 90th min it has an average percentage of 40%, whereas at 10% concentrations in the 30th min of 20% and in the 60th min of 30%, it can be seen in Figure 3.

Table 1. Colonies of Ungrowth Bacteria on Time Evaporation of Lavender Aromatherapy with Concentration Variation

Concentration of Aromatherapy	The number of bacterial colonies that ungrowth after evaporation		
	30th Min	60th Min	90th Min
5%	1 (bacteria No.4)	1 (bacteria No.4)	-
7.5%	1 (bacteria No.4)	2 (bacteria No.4 and No.7)	-
10%	1 (bacteria No.4)	1 (bacteria No.4)	1 (bacteria No.4)

Table 2. Colonies of Ungrowth Bacteria on Time Evaporation of Rosemary Aromatherapy with Concentration Variation

Concentration of Aromatherapy	The number of bacterial colonies that ungrowth after evaporation		
	30th Min	60th Min	90th Min
5%	-	-	-
7.5%	-	-	1 (bacteria No.4)
10%	2 (bacteria No.4 and No.9)	-	-

Table 3. Colonies of Ungrowth Bacteria on Time Evaporation of Ylang-ylang Aromatherapy with Concentration Variation

Concentration of Aromatherapy	The number of bacterial colonies that ungrowth after evaporation		
	30th Min	60th Min	90th Min
5%	1 (bacteria No.4)	1 (bacteria No.4)	-
7.5%	1 (bacteria No.4)	1 (bacteria No.4)	4 (bacteria No.4,5,7,9)
10%	2 (bacteria No.4, 9)	3 (bacteria No.4,5, 9)	1 (bacteria No.4)

Table 4. Gram Staining of Positive Coccus Bacteria and Positive Bacillus on Evaporation of Lavender, Rosemary and Ylang-lang Aromatherapy with Concentration and Evaporation Time Variation

Essensial oil	Concentration of Aromatherapy	30th Min		60th Min		90th Min	
		Positive Coccus	Positive Bacillus	Positive Coccus	Positive Bacillus	Positive Coccus	Positive Bacillus
Lavender	5%	-	1	-	1	-	-
	7.5%	-	1	-	2	-	-
	10%	-	1	-	1	-	1
Rosemary	5%	-	-	-	-	-	-
	7.5%	-	-	-	-	-	1
	10%	-	2	-	-	-	-
Ylang-ylang	5%	-	1	-	1	-	-
	7.5%	-	1	-	1	-	4
	10%	-	2	-	3	-	1

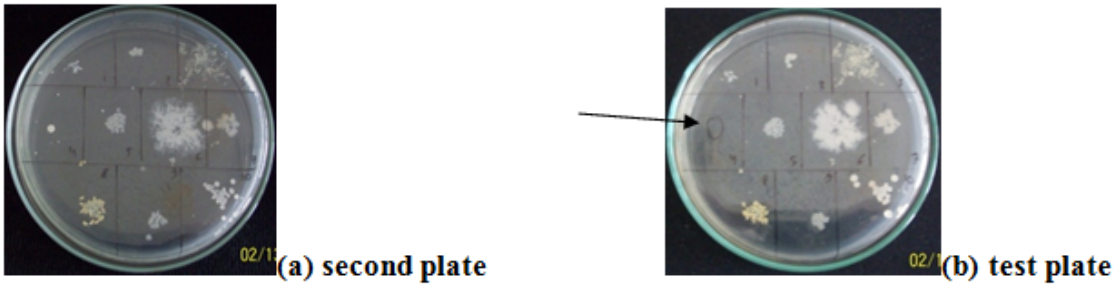

Figure 1. Colonies of bacteria before (a) and after (b) evaporation of lavender aromatherapy

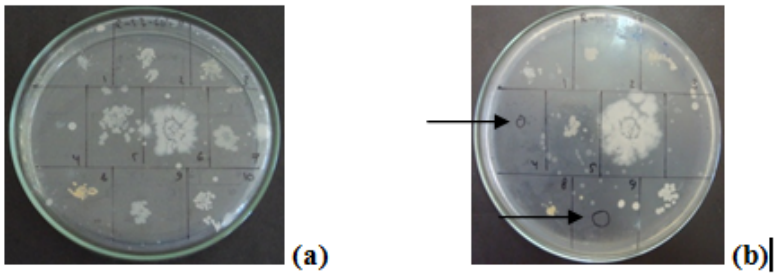

Figure 2. Colonies of bacteria before (a) and after (b) evaporation of rosemary aromatherapy

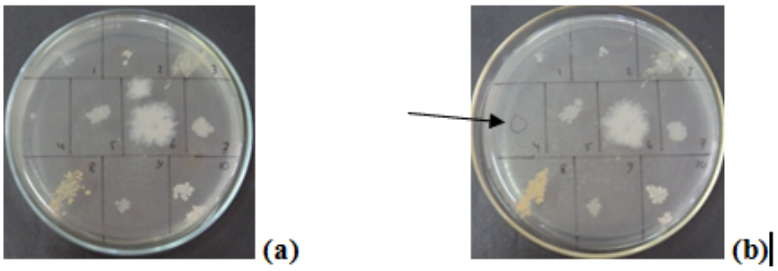

Figure 3. Colonies of bacteria before (a) and after (b) evaporation of ylang-ylang aromatherapy

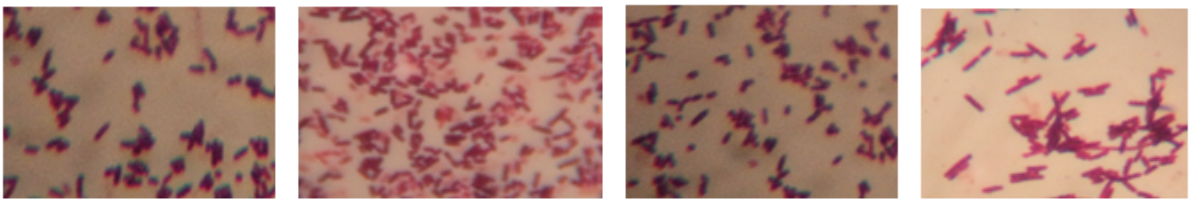

Figure 4. Form of Gram positive bacteria

The colonies of ungrowth bacteria from each variation of concentration and evaporation time, it was seen that the essential oils of lavender, rosemary, and ylang-ylang had effectiveness activities as antibacterial.

Based on the test with the variation of concentration, the result of minimal concentration for lavender and ylang-ylang essential oils was 5%, and for rosemary 7.5%. Due to not using solvents but using only a mixture of aquadest, then for a concentration of 5% consisting of 4 ml of oil and 76 ml of aquadest and for a concentration of 7.5% comprising 6 ml of oil and 74 ml of aquadest.

Each of the oils has a different concentration due to the presence of different oil content, but the essentials oil were not soluble in water but if it was heated it will evaporated and removed the aroma. Therefore, concentration was very influential in the evaporation of aromatherapy. If the concentration more and more, then the effect was greater but not applicable to all types of essential oils. There were also those with just a few drops of oil can have a greater effect, it was because each oil has different properties, namely:

a. Top note, a type of volatile oil and its aroma was only resistant for 3-4 h only. But with just a few drops of this oil can already spread the scent was very stinging.
b. Middle note, a type of oil whose aroma can last up to 3 d. This type of oil was usually used as a relaxant because it was quite durable.
c. Base note, an essential oil whose aroma can last for 3-7 d. The effect of this oil was more durable, but it was not as good as the first time. This type of oil was very popular people especially as fragrances, because the aroma was long enough.

Microscopic identification

After evaporation of lavender, rosemary, and ylang-ylang essential oils with variation of concentration for 30, 60 and 90 min, dead bacterial colonies were microbiologically identified using Gram staining with 100x magnification of the microscope has been clearly seen that a Gram positive bacteria were seen in Tables 4 and Figure 4. Although the Gram positive bacteria species exist that can form spores so that bacteria already dispersed in the air by oil vapor will not die, but with the use of lavender, rosemary, and ylang-ylang aromatherapy in a room can help kill bacterial colonies.

CONCLUSION

The minimum inhibitory concentration respectively, 5% v/v with effective duration in 60 min for lavender and ylang-ylang essential oils that be required to have the effect on reducing the population of airborne bacteria and 7.5% for rosemary with effective duration in 90 min.

REFERENCES

1. Atsumi T, Tonosaki K. Smelling lavender and rosemary increases free radical scavenging activity and decreases cortisol level in saliva. Psychiatry Research 2007; 150(1):89-96.
2. Dunning T. Applying a quality use of medicines framework to using essential oils in nursing practice. Complement 2005;11(3):172-81.
3. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res 2007;21(4):308-23.
4. Emerson J. Aromatherapy: Top Aromatherapy Essential Oils, Balms And Lotions. New York: BizDirect/iSynergyGroup. 2004.
5. Pibiria MC, Goelb A. Vahekenic N, Rouleta CA. Indoor air purification and ventilation systems sanitation with essential oils. International Journal of Aromatherapy 2006;16(3-4): 149-53.
6. Babar A, Naser A. Al-Wabel, Saiba S, Aftab A, Shah AK, Firoz A. Essential oils used in aromatherapy: A systemic review. Asian Pac J Trop Biomed 2015;5(8):601–11
7. Moss M, Cook J, Wesnes K, *et al*. Aromas of rosemary and lavender essential oils differentially affects cognition and mood in healthy adults. Int J Neurosci 2003;113:15-38.
8. Vinayak MG, Rupali N, Kiran BD, Atul NK, Kiran BK, *et al*. Aromatherapy: Art or science. International Journal of Biomedical Research 2013;04(2):74-83

9. Roberts CF. A replica plating technique for the isolation of nutritionally exacting mutants of a filamentous fungus (*Aspergillus nidulans*). J. gen. Microbiol 1959;20:540-48
10. Rao S. Gram's staining. [ONLINE]. [Cited 2016 Dec 28] Available from: http://www.microrao.com/micronotes/pg/Gram%20stain.pdf.

Cite this article as:

Insan Sunan Kurniawansyah *et al*. The antibacterial activities of aromatherapy essential oils of lavender (*Lavandula angustifolia* Mill), rosemary (*Rosmarinus officinalis* L.) and ylang-ylang (*Cananga odorata* (Lamk.) Hook) against airborne bacteria. Int. Res. J. Pharm. 2018;9(6):71-75 http://dx.doi.org/10.7897/2230-8407.09692

Source of support: Nil, Conflict of interest: None Declared