

IN VITRO ANTIMICROBIAL ACTIVITY OF SOME ESSENTIAL OILS AGAINST BACTERIAL PATHOGENS CAUSING SKIN DISEASES IN VAPOR PHASE

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ABSTRACT

Objective: The objective of this study was to evaluate the antimicrobial activity of volatile oils from aromatic plants against pathogenic bacteria.

Methods: Thai aromatic plants such as *Pogostemon cablin* (Blanco) Benth (Patchouli oil), *Cymbopogon nardus* Rendle (Citronella grass oil), *Pelargonium roseum* (Geranium oil), *Syzygium aromaticum* (L.) Merrill and Perry (clove oil), *Cinnamomum* spp.(cinnamon oil), and *Cymbopogon citratus* (DC.) Stapf. (lemongrass oil) were selected. Essential oils were obtained by water distillation and were stored at 4°C until use. Five human pathogenic bacteria were obtained from Thai traditional Medicine College, Rajamangala University of Technology, *Staphylococcus epidermidis*, *Escherichia coli*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *Pseudomonas aeruginosa*. The antibacterial activity of volatile oils was determined by disc-diffusion assay. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each essential oil were determined.

Results: Our study showed that 10% of essential oil from *Cinnamomum* spp. was the most potential against *S. aureus*, MRSA, and *E. coli* when assayed by disc-diffusion method with inhibition zones ranging from 37.66±0.57 to 45.33±1.15 mm and from 29.33±0.57 to 36.00±1.00 for lemongrass oil with MIC and MBC of 1.25%.

Conclusion: From this study, it can be concluded that some essential oils have potential antibacterial activity. The present investigation provides support to the antibacterial properties of essential oils and will be applied to health-care product as aroma antibacterial products.

Keywords: Antibacterial activity, Skin diseases, Volatile oils, Pathogen.

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INTRODUCTION

Skin infectious disease is a disorder of exclusively the superficial layers of the skin, infected by organisms such as bacteria, viruses, fungi, and parasites [1]. The pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and drug-resistant bacteria caused several skin human infections [2-8]. Thus, the global prevalence of infectious disease caused by bacteria is a major public health problem [9]. However, it is important to note that the effects of synthetic drugs can be highly unpredictable, making difficult to fully assess their physical impact. Hence, the natural compounds or essential oils from medicinal plant have an alternative to treat the antimicrobial agent. Medicinal plants have been used as primary treatment of skin disorders for centuries in Thailand Traditional Medicine, Chinese Traditional Medicine, and Indian Traditional Medicine.

Essential oils of medicinal plants present a great potential of application as natural antimicrobial agents [9]. They are mixtures of natural volatile molecule deriving from plant secondary metabolism which potent antibacterial, antifungal, antiviral, insecticidal and antioxidant activities. Generally, the biological activity of essential oil depends on their chemical structure, environmental, and agronomic conditions [10]. Thus, the objective of this research was to screen the antibacterial activity of volatile oil from medicinal plants in Thailand against skin human pathogenic bacteria.

METHODS

The extraction of essential oils from medicinal plants

Pogostemon cablin (Blanco) Benth (Patchouli oil), *Cymbopogon nardus* Rendle (Citronella grass oil), *Pelargonium roseum* (Geranium oil), *Syzygium aromaticum* (L.) Merrill and Perry (clove oil), *Cinnamomum* spp.(cinnamon oil), and *Cymbopogon citratus* (DC.)

Stapf. (lemongrass oil) were selected. Briefly, the medicinal plant was completely immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapor and finally collected after decantation. The distillate was isolated and dried in a Rotavapor to giving greenish-yellow oil. The oil was stored at 4°C until the antimicrobial screening [11].

Antibacterial activity of the essential oils

Microorganisms

Antimicrobial activity determination was carried out against bacterial pathogens causing skin diseases, *Staphylococcus epidermis*, *E. coli*, methicillin-resistant *S. aureus* (MRSA), *S. aureus*, and *P. aeruginosa*.

Culture media

Bacteria were assayed on nutrient agar (NA) (Merck, g/L):peptone from meat, 50.0; meat extract, 3.0; agar, 12.0; and water, 1.0 L.

Inocula

Inocula for the assays were prepared by incubation at 37°C for 18 h before use and were adjusted to McFarland scale of 0.5. Cell suspensions were finally diluted to 10⁴ cfu/ml for being used in the activity assay.

Disc volatilization assay

This assay requires a culture agar plate inoculated with microbial suspension adjusted by McFarland No.0.5 containing 10⁶ cfu/ml inserted down on top of a container (Fig. 1). A paper disc (6 mm) is deposited at the bottom of the container with 15 µl of essential oils. All plates inoculated and the disc should be sealed with parafilm to prevent the steam outlet and incubated at 37°C for 24 h, and the inhibition zone was measured in millilitre (mm) [12]. The experiments were done by three replicates (Fig. 1).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

To assess the MIC and MBC of essential oils, the serial two-flow dilution was carried out with the disc-diffusion assay. The MIC represented the lowest concentration showing an inhibition zone; the MBC was determined by subculturing which showed no bacterial growth on the agar plates after incubated at 37°C for 24 h in an incubator. The lowest concentration that did not show bacterial growth was defined

as an MBC value. The experiment was conducted in triplicate, and the mean diameter of the zone of inhibition was recorded in millimeters (mm). Erythromycin was used as a positive control, and dimethyl sulfoxide (DMSO) solution was used as a negative one. All experiments were performed in triplicate. The results were represented by mean±standard deviation (SD) [13,14].

Statistical analysis

The results of the data experiments were expressed in mean±SD for groups ($n=3$).

RESULTS AND DISCUSSION

Antibacterial activity of essential oils

Due to the absence of direct contact between the skin pathogenic bacteria and essential oils, the common skin pathogenic bacteria are listed in Table 1. The MIC was determined only for oils that presented positive on disc-diffusion assay. The results show a variable effect of essential oils on the bacterial pathogens. The essential oils from *Cinnamomum* spp. was great potential against MRSA, showing the lowest MIC values of 1.25% with clearing zone of 45.33 ± 1.15 mm and MBC value of 1.25%. As it can be appeared in Table 1, the essential oil of cinnamon oil exhibited the growth of *E. coli* and *S. aureus* with MIC and MBC value of 1.25% and showed zone of inhibition of 20.00 ± 0.00 and 37.66 ± 0.57 mm, respectively. The essential oil with the lowest MIC and MBC was that of clove, with MIC and MBC of 5.0% against *P. aeruginosa* with halo zone of 44.33 ± 1.15 mm (Table 2). The MIC and MBC of clove oil at a concentration of 1.25% showed an inhibition zone of 33.66 ± 1.52 mm with *B. subtilis*. However, as the same condition, it did not inhibit the growth of *E. coli*, *S. aureus* (MRSA) and *S. aureus*. The MIC and MBC of lemongrass oils at a concentration of 2.50% displayed the lowest concentration to inhibit the growth of MRSA, *S. aureus*, and *B. subtilis* with clearing zone of 36.00 ± 1.00 , 36.00 ± 1.00 and 28.33 ± 1.52 mm (Fig. 2). It also no antibacterial activity was observed in the controls as well as in the assays where only DMSO was used. Above 5.0%, patchouli oil, citronella oil, and geranium oil did not show any antibacterial effective against *E. coli*, *S. aureus* (MRSA), *S. aureus*, and *P. aeruginosa*. In contrary, the citronella oil showed potent inhibition against pathogens [13]. The vaporization of the inhibition zone of cinnamon oil was observed on NA with the difference various skin pathogens and concentration of essential oils. This result was similar of the previous reported, concerning the antioxidant and the antimicrobial activity [15-20], indicating that the different plants species have a variety of different kinds of molecules. Hence, differences in the composition of essential oils, in the interactions of their constituents, and in their extraction and purification operations affect their antimicrobial activity [21].

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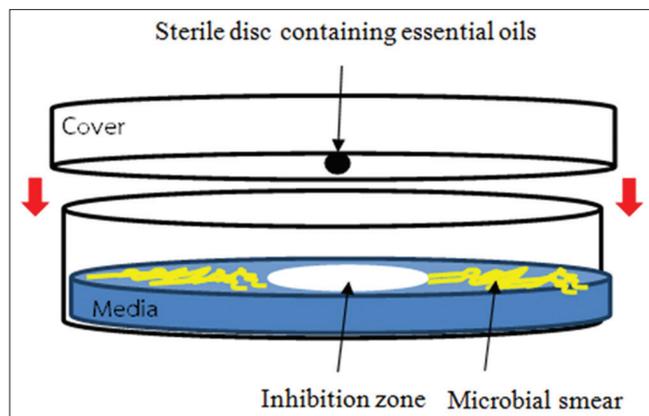


Fig. 1: Disc volatilization assay

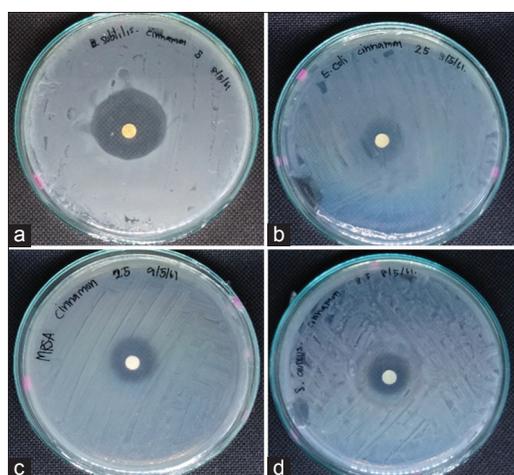


Fig. 2: The effect of cinnamon oil against skin pathogens. (a) *Bacillus subtilis* (5 mg/ml), (b) *Escherichia coli* (2.5 mg/ml), (c) *Staphylococcus aureus* (methicillin-resistant *S. aureus*) (2.5 mg/ml), (d) *S. aureus* (2.5 mg/ml)

Table 1: The minimum inhibitory concentration of essential oils against skin pathogens

Essential oils	Microorganisms									
	<i>Escherichia coli</i>		MRSA		<i>Staphylococcus aureus</i>		<i>Pseudomonas aeruginosa</i>		<i>Bacillus subtilis</i>	
	MIC ^a	Clear zone ^b	MIC ^a	Clear zone ^b	MIC ^a	Clear zone ^b	MIC ^a	Clear zone ^b	MIC ^a	Clear zone ^b
Patchouli oil	-	-	-	-	-	-	-	-	-	-
Citronella oil	-	-	-	-	-	-	-	-	5.00	12.33 ± 0.57
Geranium oil	-	-	-	-	-	-	-	-	-	-
Lemongrass oil	-	-	2.50	36.00 ± 1.00	2.50	36.00 ± 1.00	-	-	2.50	28.33 ± 1.52
Clove oil	-	-	-	-	-	-	5.00	44.33 ± 1.15	1.25	33.66 ± 1.52
Cinnamon oil	1.25	20.00 ± 0.00	1.25	45.33 ± 1.15	1.25	37.66 ± 0.57	-	-	-	-
(+) control (10 mg/ml)	ND	36.33 ± 1.15	ND	ND	ND	37.33 ± 0.57	ND	44.00 ± 1.00	ND	35.66 ± 0.57
(-) control ^c	-	-	-	-	-	-	-	-	-	-

^aMIC (%), ^bClearing zone of inhibition in terms of (mm), ^cDMSO (5%). -: No inhibition, ND: Not detected, MRSA: Methicillin-resistant *Staphylococcus aureus*, DMSO: Dimethyl sulfoxide, MIC: Minimum inhibitory concentration

Table 2: The minimum bactericidal concentration of essential oils against skin pathogens

Essential oils	Microorganisms				
	<i>Escherichia coli</i>	MRSA	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>
	MBC ^a	MBC ^a	MBC ^a	MBC ^a	MBC ^a
Patchouli oil	-	-	-	-	-
Citronella oil	-	-	-	-	5.0
Geranium oil	-	-	-	-	-
Lemongrass oil	-	2.50	2.50	-	2.50
Clove oil	-	-	-	5.0	1.25
Cinnamon oil	1.25	1.25	1.25	-	-

^aMBC (%). MRSA: Methicillin-resistant *Staphylococcus aureus*, MBC: Minimum bactericidal concentration

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