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Original Article

Antimicrobial Activity of Essential Oils Against Five Strains of *Propionibacterium acnes*

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Abstract The present study was conducted to evaluate the *in vitro* antibacterial activities of 22 essential oils from Thai medicinal plants against 5 strains of *Propionibacterium acnes* (*P. acnes*). Antibacterial activity of essential oils was investigated using disc diffusion and agar dilution methods. The results showed that 19 oils could inhibit the growth of *P. acnes*. According to the inhibition zone, kaffir lime leaf (*Citrus hystrix* DC.), lemongrass oil (*Cymbopogon citratus* (DC.) Stapf), clove oil (*Syzygium aromaticum* (L.) Merr. & Perry) and michelia oil (*Michelia alba* DC.) had the strongest antibacterial activity. The results form the agar dilution method showed the same trend in which lemongrass oil and citronella oil showed the lowest minimum inhibitory concentration against 5 strains bacteria at 0.125% v/v. Therefore, these two essential oils are an interesting source for further study and possibly as an alternative acne treatment.

Keywords: acne vulgaris, antibacterial activity, essential oils, *Propionibacterium acnes*

INTRODUCTION

Acne vulgaris is an inflammatory skin disease of pilosebaceous units. *Propionibacterium acnes* (*P. acnes*) plays an important role in the pathogenesis of acne inflammation by producing polymorphonuclear leukocyte and monocyte or macrophage to produce pro-inflammatory mediators.[1] Moreover, *P. acnes* can also induce follicular keratinocytes to release interleukin-1, which causes keratinocytes to proliferate and contributes to the formation of the preclinical micromedo.[2] Therefore, the compounds for targeting acne vulgaris should be able to inhibit *P. acnes*. For many years, antibiotics have been used to treat acne vulgaris; however, the presence of antibiotic resistance strains has been increasing as shown in many reports.[3-6] Hence, there is a challenge for the discovery of new substances from natural sources to overcome this problem.

Essential oils are complex natural mixtures that are isolated by steam distillation. The majority of them are used as flavoring agents in perfume, food and beverage. Additionally, they have been traditionally used as natural preservatives in medicine, food and cosmetics. Moreover, several reports have indicated antibacterial activities of essential oils.[7-15] Therefore, researchers are interested in searching for essential oils from medicinal plants which have been traditionally used as antibacterial agents by examining against the microorganism predominately involved in acne inflammation, *P. acnes*.

MATERIALS AND METHODS

Materials

Samples of pure essential oils with known compositions which were obtained via steam distillation were purchased from Thai China Flavors & Fragrances Industry Co. (Thailand).

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The test organisms used in this study were *P. acnes* (DMST No. 14916, 14917, 14918, 21823, 21824). These bacteria were obtained from Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand.

Disc Diffusion Method

This experiment was performed by following the method of Hayes and Markovic (2002)[1] with some modifications. Briefly, *P. acnes* was incubated in Brain heart infusion broth supplemented with 1% glucose and adjusted to yield approximately 1.0 x 10^8 CFU/ml. The inoculum suspension was swabbed on the entire surface of brain heart infusion agar containing 1% glucose and 0.5% polysorbate 80. Sterile filter paper discs (Macherey-Nagel, MN®) were aseptically placed on inoculated plate and impregnated with 15 µl of each essential oil. The plates were left at ambient temperature for 30 minutes to allow exceed prediffusion prior to incubation at 37°C for 72 hours under anaerobic conditions using the gas generation kit (Oxoid, UK). Standard discs of tetracycline (30 µg/disc), erythromycin (15 µg/disc), and clindamycin (2 µg/disc) (Oxoid, English) were used individually as positive controls. The diameters of the inhibition were measured (mm) and recorded as the mean ± S.D. (standard deviation). All experiment was performed in duplicate and repeated three times.

Agar Dilution Method

The minimal inhibitory concentration (MIC) values were determined by agar dilution method. Test materials were added aseptically to 20 ml aliquots of appropriate sterile molten agar containing 0.5% polysorbate 80 at the appropriate volumes to produce the required concentration range of test material (0.015-4.0% v/v). The resulting agar solutions were vortexed at high speed for 15 seconds or until completely dispersed, immediately poured into sterile petri dishes then allowed to set for 30 minutes. The plates were then spotted inoculated with multipoint inoculator to transfer the desired test organisms (1 µl) from the prepared inoculum onto the plates. Inoculated plates were left until the inoculum had set and then incubated at 37°C for 72 hours under anaerobic condition as previous described. Following the incubation period, the plates were observed and recorded for presence or absence of growth. From the results, the MIC was recorded as the lowest concentration of test materials where absence of growth was observed.

RESULTS AND DISCUSSION

The present results indicated that 19 essential oils could effectively inhibit the growth of *P. acnes.* Among these essential oils, kaffir lime leaf (*Citrus hystrix* DC.), lemongrass oil (*Cymbopogon citratus (*DC.) Stapf), clove oil (*Syzygium aromaticum* (L.) Merr. & Perry) and michellia oil (*Michelia alba* DC.) showed the strongest antibacterial activity with inhibition zones of more than 20 mm (Table 1). Subsequent experiments were conducted to determine inhibitory concentrations of all essential oils. Lemongrass and citronella oil (*Cymbopogon nardus* L.) revealed the highest antibacterial effect as they possessed the MIC value of 0.125% v/v for all of 5 strains bacterial tested (Table 2). Even though kaffir lime leaf (*Citrus hystrix* DC.) showed the largest inhibition zone, its MIC value was greater than that of lemongrass and citronella oil. This may be due to their abilities to diffuse from paper disc and solubilize in agar; however, the disc diffusion method was used for screening the antibacterial activity of the essential oils.[16,17]

Lertsatithanakorn *et al.*[18] reported that the MICs against *P. acnes* of lemongrass, kaffir lime, holy basil and plai, determined by a broth microdilution method, were 0.6 µl/ml, 5.0 µl/ml, 5.0 µl/ml and 25.0 µl/ml, respectively. The lower MICs from that study may be due to the different method used.

Generally, essential oils comprise of a large number of active components. The difference in antibacterial activity of the essential oils may be due to the difference in chemical compositions. The main components of essential oils exhibited high activity in this study were previously reported for their

marked antibacterial activity against various types of bacteria. Citral, a main component in lemongrass oil, showed activity against several microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*.[12,19-21] Similarly, geraniol, trans-citral, cis-citral, geranyl acetate, citronellal and citronellol in citronella oil[22]

Table 1. Antibacterial activity of essential oils against 5 strains of *Propionibacterium acnes*

Test samples	Inhibition zone (mm)[a] ± S.D.				
	P. acnes DMST 14916	*P. acnes* DMST 14917	*P. acnes* DMST 14918	*P. acnes* DMST 21823	*P. acnes* DMST 21824
Black pepper oil (*Piper nigrum* L.)	-	-	-	-	-
Canaga abs oil (*Cananga odorata* Hook.f. & Thomson var. *fruticosa* (Craib) Corner)	-	-	-	-	-
Citronella oil (*Cymbopogon nardus* L.)	18.1± 0.6	18.1± 0.5	17.9 ± 0.4	18.4 ± 0.8	19.5 ± 0.5
Clove oil (*Syzygium aromaticum* (L.) Merr. & Perry)	25.3 ± 2.5	23.6 ± 4.3	21.8 ± 2.1	25.3 ± 1.3	23.3 ± 0.3
Coriander oil (*Coriandrum sativum* L.)	13.7 ± 2.2	12.8 ± 2.2	11.6 ± 1.4	9.9 ± 1.5	12.3 ± 2.2
Eucalyptus oil (*Eucalyptus globulus* Labill.)	-	-	-	-	-
Galanga oil (*Alpia galangal* (L.) Swatz)	8.6 ± 0.8	9.4 ± 1.4	9.6 ± 1.2	9.8 ± 2.0	8.9 ± 0.7
Ginger oil (*Zingiber offinale* Roscoe)	9.6 ± 0.7	9.6 ± 1.5	8.9 ± 0.1	9.0 ± 0.4	9.0 ± 0.7
Guava leaf oil (*Psidium guajava* L.)	13.1 ± 0.5	12.5 ± 1.5	11.6 ± 1.1	14.7 ± 1.8	12.4 ± 1.9
Holy basil oil (*Ocimum tenuiflorum* L.)	16.5 ± 3.3	17.3 ± 1.4	17.3 ± 3.8	16.3 ± 3.6	17.2 ± 3.7
Jasmine oil (*Jasminum sambac* Ait.)	12.8 ± 0.6	12.0 ± 1.2	11.3 ± 0.8	12.9 ± 1.4	12.8 ± 1.0
Kaffir lime oil (*Citrus hystrix* DC.)	14.1 ± 3.0	18.0 ± 1.4	16.7 ± 1.5	15.1 ± 2.6	17.3 ± 0.4
Kaffir lime leaf oil (*Citrus hystrix* DC.)	> 90	> 90	> 90	> 90	> 90
Lavender oil (*Lavandula angustifolia*)	17.8 ± 1.1	17.7 ± 2.2	17.8 ± 2.0	20.2 ± 2.3	15.5 ± 2.8
Lemongrass oil (*Cymbopogon citratus* (DC.) Staphf)	35.8 ± 3.9	34.4 ± 2.9	35.6 ± 4.1	31.9 ± 3.9	36.9 ± 3.8
Lesser galangal (*Alpinia officinarum* Hance)	14.8 ± 0.2	14.6 ± 0.8	15.3 ± 0.3	13.6 ± 2.7	15.0 ± 2.7
Michellia oil (*Michelia alba* DC.)	23.8 ± 1.6	21.5 ± 2.9	22.7 ± 2.3	28.3 ± 4.6	22.0 ± 3.0
Plai oil (*Zingiber montanum* (Koenig) Link ex Dietr.)	13.5 ± 2.0	13.9 ± 0.9	14.7 ± 1.7	13.3 ± 0.7	15.7 ± 2.2
Sweet basil oil (*Ocimum basilicum* L.)	11.7 ± 0.7	10.4 ± 1.3	10.4 ± 2.0	10.9 ± 1.8	10.5 ± 0.6
Tea tree oil (*Melaleuca alternifolia*)	16.2 ± 1.2	15.2 ± 1.7	15.6 ± 2.6	17.4 ± 1.7	15.3 ± 1.9
Tumeric oil (*Curcuma longa* L.)	10.3 ± 0.4	11.0 ± 0.1	9.6 ± 0.7	10.1 ± 1.5	9.3 ± 0.9
Ylang ylang oil (*Cananga odorata* (Lam.) Hook. f. & T. Thomson var. *odorata*)	8.8 ± 0.7	9.4 ± 0.1	9.5 ± 0.7	9.4 ± 0.1	9.2 ± 0.1
Clindamycin (2 µg/disc)	31.0 ± 1.3	31.2 ± 1.5	30.4 ± 1.6	29.4 ± 0.8	29.1 ± 1.7
Erythromycin (15 µg/disc)	30.9 ± 1.4	31.3 ± 2.8	29.1 ± 1.3	29.2 ± 1.1	28.6 ± 1.5
Tetracycline (30 µg/disc)	35.5 ± 4.6	35.3 ± 3.1	35.5 ± 3.8	35.3 ± 3.0	35.8 ±2.8

[a] Values for inhibition zone are presented as mean ± S.D. of three independent experiments.
- Inhibition zone was not more than 6 mm (diameter of disc).

Table 2. The minimum inhibitory concentration values of essential oils against 5 strains of *Propionibacterium acnes*

Test samples	Minimum inhibotory concentration (% v/v)				
	P. acnes DMST 14916	*P. acnes* DMST 14917	*P. acnes* DMST 14918	*P. acnes* DMST 21823	*P. acnes* DMST 21824
Black pepper oil (*Piper nigrum* L.)	> 4	> 4	> 4	> 4	> 4
Canaga abs oil (*Cananga odorata* Hook.f. & Thomson var. *fruticosa* (Craib) Corner)	> 4	> 4	> 4	> 4	> 4
Citronella oil (*Cymbopogon nardus* L.)	0.125	0.125	0.125	0.125	0.125
Clove oil (*Syzygium aromaticum* (L.) Merr. & Perry)	0.25	0.25	0.25	0.25	0.25
Coriander oil (*Coriandrum sativum* L.)	1	1	1	1	1
Eucalyptus oil (Eucalyptus globulus Labill.)	4	4	4	4	4
Galanga oil (*Alpia galangal* (L.) Swatz)	> 4	> 4	> 4	> 4	> 4
Ginger oil (*Zingiber offinale* Roscoe)	> 4	> 4	> 4	> 4	> 4
Guava leaf oil (*Psidium guajava* L.)	> 4	> 4	> 4	> 4	> 4
Holy basil oil (*Ocimum tenuiflorum* L.)	2	2	2	2	2
Jasmine oil (*Jasminum sambac* Ait.)	2	2	2	2	2
Kaffir lime oil (*Citrus hystrix* DC.)	2	2	2	2	2
Kaffir lime leaf oil (*Citrus hystrix* DC.)	0.25	0.25	0.25	0.25	0.25
Lavender oil (*Lavandula angustifolia*)	2	2	2	2	2
Lemongrass oil (*Cymbopogon citratus* (DC.) Staphf)	0.125	0.125	0.125	0.125	0.125
Lesser galanga (*Alpinia officinarum* Hance)	1	1	1	1	1
Michellia oil (*Michelia alba* DC.)	1	1	1	1	1
Plai oil (*Zingiber montanum* (Koenig) Link ex Dietr.)	4	4	4	4	4
Sweet basil oil (*Ocimum basilicum* L.)	> 4	> 4	> 4	> 4	> 4
Tea tree oil (*Melaleuca alternifolia*)	1	1	1	1	1
Tumeric oil (*Curcuma longa* L.)	> 4	> 4	> 4	> 4	> 4
Ylang ylang oil (*Cananga odorata* (Lam.) Hook. f. & T. Thomson var. odorata)	> 4	> 4	> 4	> 4	> 4

and eugenol, β-caryophyllene and acetyl-eugenol in clove oil[23] also showed activities against various microorganism such as *S. aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Clostridium perfringens* and *E. coli*.[13-15] Therefore, antibacterial activity against *P. acnes* of these essential oils may be due to the activity of those key constituents.

CONCLUSION

As previously mentioned, compounds exhibiting activity against *P. acnes* can be used to treat acne vulgaris. Lemongrass oil and citronella grass oil had the highest inhibitory activity against *P. acnes* by which their MIC values were the same, *i.e.* 0.125% v/v. Therefore, these two essential oils may have a potential for further study as an alternative acne treatment.

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