ORIGINAL ARTICLE

Formulation of anti-acne concealer containing cinnamon oil with antimicrobial activity against *Propionibacterium acnes*

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ABSTRACT

This study aimed to develop an anti-acne concealer containing essential oil with anti-*Propionibacterium acnes* activity. Antimicrobial activity of cinnamon oil, galangal oil, and eucalyptus oil against *P. acnes* DMST 14916 was assayed using agar disc diffusion and the broth dilution method. Cinnamon oil showed the maximum inhibitory activity against *P. acnes* with a clear zone diameter of 36.75 ± 1.06 mm, compared to 14.67 ± 0.58 and 41.83 ± 1.04 mm for tea tree oil and clindamycin, respectively. The minimum inhibitory concentration value of cinnamon oil was 5 mg/mL. Among five formulations of concealer incorporating 0.5% w/w cinnamon oil, F4 provided good texture, coverage, and spreadability with anti-*P. acnes* activity and stable under determined storage conditions. Cinnamon oil could be a promising cosmetic ingredient for anti-acne products, and F4 concealer may be useful for both covering skin imperfections and the management of acne.

Key words: Anti-acne, cinnamon oil, concealer, *Propionibacterium acnes*

INTRODUCTION

Acne is a common dermatological disease that mainly affects adolescents. It can lead to both physical and psychological effects, resulting in depression, poor self-esteem, and suicidal tendencies.[1] Acne vulgaris is diagnosed by the presence of various clinical manifestations such as closed and open comedones, papules, pustules, cysts, and nodules.[2] The underlying pathogenesis of these lesions is multifactorial and includes high sebum secretion, hyperkeratinization, hormonal changes, and bacterial infection.[3] *Propionibacterium acnes* plays a significant role in the pathogenesis of acne. This bacterium can activate certain inflammatory mediators and metabolize sebaceous triglycerides into fatty acids, which induce the attraction of white blood cells to the plugged follicle, leading to skin inflammation. When the wall of the hair follicle is broken down, sebum, dead cells, and bacteria are secreted leading to a spectrum of acne severity.[4] Current treatments of acne include topical therapies (comedolytic agents, antibiotics, and anti-inflammatory drugs) and systemic therapies (antibiotics, zinc, and hormones).[5,6] However, these drugs cause various potential side effects, and long-term treatment with antibiotics may lead to the antibiotic resistance among acne-causing bacteria.[5]

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The study was to evaluate the antimicrobial activity of cinnamon oil, galangal oil, and eucalyptus oil against *P. acnes*, compared to tea tree oil and clindamycin. The most effective volatile oil was incorporated into a silicone-based concealer, and the best formulation was examined for physical, chemical, and microbial stabilities.

MATERIALS AND METHODS

Chemicals

P. acnes DMST 14916 were purchased from the National Institutes of Health, Thailand. The study was approved by the Institutional Biosafety Committee, Naresuan University, Thailand. The ingredients of the formulation were purchased from Namsiang Trading Co., Ltd., Thailand. Essential oils, kojic dipalmitate, and dimethicone were purchased from Chemipan Corporation Co., Ltd., Thailand. Clindamycin was obtained from Pfizer international Co., Ltd. Brain–heart infusion (BHI) agar and BHI broth were purchased from Merck, Germany.

Culture methods

P. acnes DMST 14916 was stored on BHI agar in an anaerobic jar at 37°C for 48 h. A suspension of *P. acnes* in BHI broth was prepared. The inoculated broths were adjusted to 0.5 McFarland standard turbidity with an approximate optical density (OD) of 0.08–0.13 at 600 nm.

Agar disc diffusion assay

Three hundred microliters of *P. acnes* suspension (1.5×10^8 CFU/mL) was spread on BHI agar. Paper discs (6.0 mm diameter) were impregnated with 20 µL of the tested samples, subsequently being placed and gently pressed on BHI agar. Clindamycin (1% w/v) was used as a positive control, whereas distilled water was used as a negative control. The inoculated plates were incubated at 37°C for 48 h under the anaerobic system. After incubation, the clear zone diameter was measured by Vernier Caliper.[7] To determine the antimicrobial activity of the concealer against *P. acnes*, concealer (0.5 g) was applied to the agar well instead of the paper disc.

Broth dilution method

Various concentrations (0.25%, 0.5%, 1.0%, and 2.0% w/v) of essential oils were prepared by 2% w/v dimethylsulfoxide (DMSO) and 2% w/v Tween-80 as a solubilizing agent, diluted with BHI broth. Three hundred microliters of *P. acnes* suspension (1.5×10^8 CFU/mL) was mixed with 2 mL of tested oils. Liquid paraffin was added and each tube was sealed tightly with Parafilm before being incubated at 37°C for 48 h in the anaerobic jar. The minimum inhibitory concentration (MIC) values were obtained from the lowest concentration of tested samples that inhibited bacterial growth in which the clear mixture was observed.[7] Clindamycin (1% w/v) was used as a positive control, whereas BHI broth containing solubilizing agent was used as a negative control. The result was confirmed by determination of OD values of the tested mixture using a microplate reader at 600 nm. The OD value was read promptly after mixing and 48 h postincubation. The percentage of growth inhibition was calculated as follows:

$$\%\ Inhibition = \left(1 - \frac{\left(\left(OD_{sample\ 48\ h} - OD_{sample\ 0\ h} \right) - \left(OD_{blank\ 48\ h} - OD_{blank\ 0\ h} \right) \right)}{\left(\left(OD_{control\ 48\ h} - OD_{control\ 0\ h} \right) - \left(OD_{blank\ 48\ h} - OD_{blank\ 0\ h} \right) \right)} \right) \times 100$$

where sample: oil in BHI broth with *P. acnes*, blank: oil in BHI broth, and control: BHI broth with *P. acnes*. The MIC_{50} and MIC_{90} values were defined as the minimum concentration necessary to achieve ≥50% and ≥90% inhibition of growth, respectively.

Gas chromatography–mass spectrometry analysis

The chemical analysis was done by gas chromatography (GC) (Hewlett Packard, Hewlett Manufacturing Co., USA) equipped with a HP-5MS column (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed as isothermal at 40°C for 1 min, then raised to 250°C at a rate of 6°C/min, and held at this temperature for 4 min. Helium was used as a carrier gas at the rate of 1.0 mL/min. The scan range was 40–500 amu.[8] Cinnamaldehyde was designated as a marker in cinnamon oil and identified by comparing the retention indices of the peaks at 13.4 min. To evaluate the cinnamaldehyde content in the formulation, a mixture of 0.5 g concealer and 5 mL hexane was shaken thoroughly before being sonicated for 15 min and filtered through a 0.45-micron filter. The filtrate was collected for GC-mass spectrometry (MS) analysis.

Formulation of concealer

We expected to formulate a stable water-in-silicone emulsion concealer, providing a viscous texture, good coverage, and spreadability with anti-*P. acnes* activity for yellow skin tones. Five formulations of concealer, labeled as F1-F5, containing 0.5% w/w cinnamon oil were prepared. The content of dimethicone, wax, pigments, and distilled water was optimized. Titanium dioxide and pigments were uniformly blended and then mixed with the mixture of various dimethicone. Kojic dipalmitate was dissolved in mineral oil, and waxes were then mixed with the dimethicone mixture before heating to 70°C. Magnesium aluminum silicate and propylene glycol were mixed in water and heated to 75°C. Silicone-based concealer was prepared by the addition of the aqueous phase to the lipophilic phase and stirred continuously. Emulsification was achieved by homogenization at 3500 rpms and volatile oil was added after the preparation had cooled down. The formulation which met our specific requirements was selected for stability testing.

Stability testing

The formulation was kept at room temperature (25°C ± 2°C) for 30 days or under heating/cooling cycle between 4°C and 45°C for 6 cycles. At the end of the storage time, the physical changes, including color, odor, separation, spreadability, and coverage properties, were determined by visual observation, and viscosity was measured using a rheometer (Brookfield® Model DV-III, Brookfield AMETEK, USA). The pH was determined using a pH meter, and cinnamaldehyde content was evaluated using GC-MS. Microbial contamination was performed using the streak plate technique.

Data analysis

All experiments were repeated three times. The results were represented as mean ± standard deviation and were analyzed using one-way analysis of variance with the threshold for statistical significance set at the level of $P < 0.05$.

RESULTS AND DISCUSSION

Antimicrobial activity of various volatile oils against *Propionibacterium* acnes

Various herbs and naturally derived compounds have been reported to exhibit anti-acne activity.[6] Tea tree oil is widely used in acne care products. It is effective against various strains of *P. acnes* and has anti-inflammatory activity.[9] Terpinen-4-ol has been reported to be its main component responsible for these activities.[10] Cinnamon has been used for the treatment of various diseases such as diabetes, inflammation, and acne.[11] The constituents of cinnamon oil from *Cinnamomum zeylanicum* bark are mainly composed of cinnamaldehyde and eugenol.[12] Lesser galangal oil is obtained from the rhizome of *Boesenbergia pandurata* (*Roxb.)* Schltr. which has been reported for anti-inflammatory, antitumor, and antidiarrheal activities.[13] The major compounds of galangal oil composed of γ-terpinene, geraniol, camphor, β-ocimene, 1,8-cineole, myrcene, and borneol.[14] Eucalyptus oil is obtained from the fresh leaves of *Eucalyptus globulus*, and 1,8-cineole has been reported to be an active ingredient.[15] It has been shown to possess antibacterial, anti-inflammatory activity[16] and facilitating wound healing.[4]

As seen in Table 1 and Figure 1, all tested oils showed the formation of clear zones which was more than that from tea tree oil but significantly less than clindamycin solution, indicating their antibacterial activity that was capable of inhibiting or slowing the growth of *P. acnes*. The maximum inhibition zone was observed with cinnamon oil, followed by galangal oil and eucalyptus oil, respectively. Antimicrobial activity of the combinations of the two most effective essential oils was assayed. Mixing a high ratio (by weight) of galangal oil and cinnamon oil led to a reduction of the clear zone. Therefore, cinnamon oil was selected for further study.

As shown in Table 2, the lowest concentration of cinnamon oil that was associated with the absence of visually observed microbial growth was 0.5% w/v. The MIC_{50} and MIC_{90} values of cinnamon oil were 0.25% w/v and 1.0% w/v, respectively. Based on these findings, 0.5% w/w cinnamon oil was incorporated in the formulation. These results were in accordance with many previous studies indicating that cinnamon oil was a potent antimicrobial agent against various microbial strains including *Staphylococcus epidermidis*, *P. acnes*,[5] and multidrug-resistant strains of *Pseudomonas aeruginosa*.[11]

GC-MS analysis

GC-MS analysis of cinnamon oil is presented in Figure 2. The major compounds were cinnamaldehyde (29.0%, 13.40 min), benzyl benzoate (19.9%, 23.42 min), and linalool (11.9%, 9.38 min). Previous research had found cinnamon bark oil to be rich in trans-cinnamaldehyde, with antimicrobial effects;[17] the acrolein group in the molecules was shown to be essential for these beneficial effects.[18] The antibacterial mechanisms of cinnamon oil have been discussed previously.[19]

Formulation of concealer

Cyclopentasiloxane (and) PEG/PPG-18/18 dimethicone was used as an emulsifier, silicone wax C30-45 alkyl dimethicone was used as a thickening agent, candelilla wax and beeswax were used as stiffening agents, and the silicon-based polymer dimethicone was used to improve skin smoothness. A list of ingredients in F1–F5 formula

Table 1: Anti-*Propionibacterium acnes* activity of essential oils by agar disc diffusion assay

Essential oil	Clear zone diameter (mm)
Eucalyptus oil	16.00±0.02
Galangal oil	19.50±0.71
Cinnamon oil	36.75±1.06
Cinnamon oil: galangal oil (75:25)	34.25±1.05
Cinnamon oil: galangal oil (50:50)	31.75±1.06
Cinnamon oil: galangal oil (25:75)	27.00±0.01
Tea tree oil	14.67±0.58
Clindamycin solution	41.83±1.04
Distilled water	0.00±0.00

Table 2: Percentage inhibition of cinnamon oil against *Propionibacterium acnes* by broth dilution assay

Concentration (percentage w/v)	Percentage inhibition
0.25	56.92±2.15
0.50	88.08±7.02
1.0	96.92±6.21
2.0	96.92±4.18

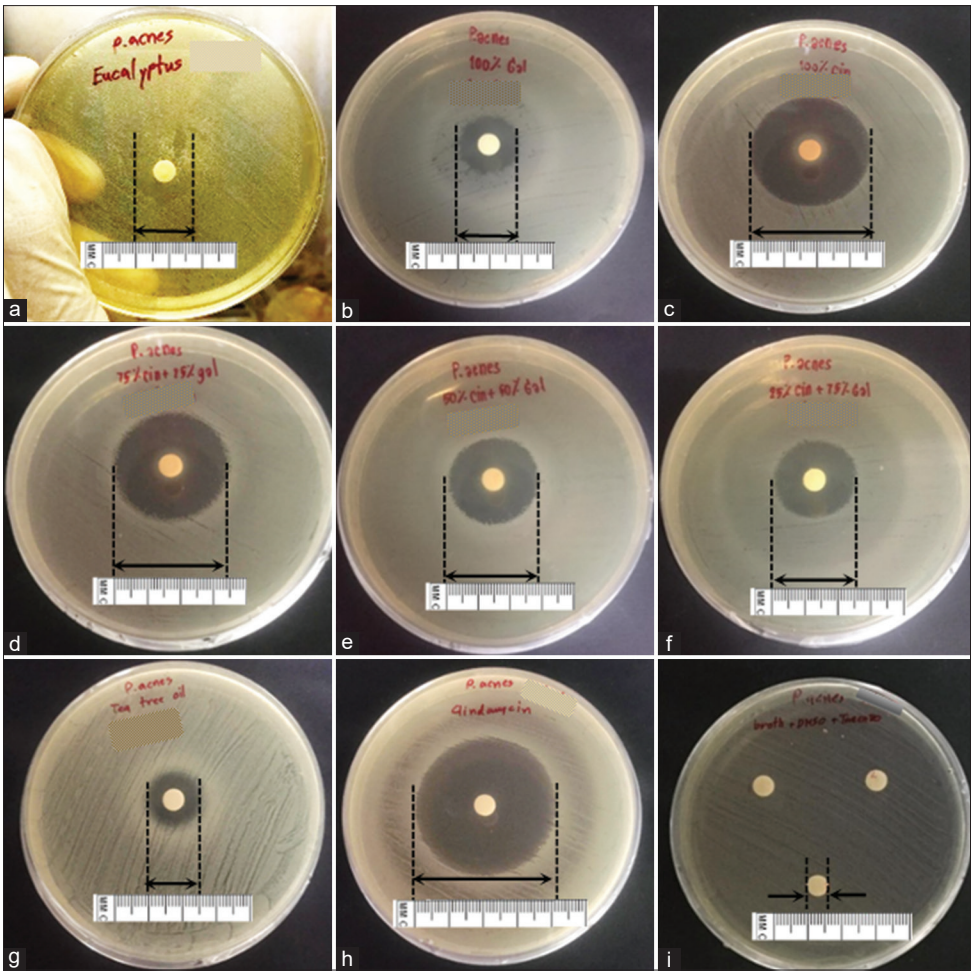

Figure 1: Inhibition zone of (a) eucalyptus oil, (b) galangal oil, (c) cinnamon oil and mixture of cinnamon oil and galangal oil at ratio (d) 75:25, (e) 50:50, (f) 25:75, compared with (g) tea tree oil, (h) clindamycin and (i) distilled water against *P. acnes* by agar disc diffusion assay

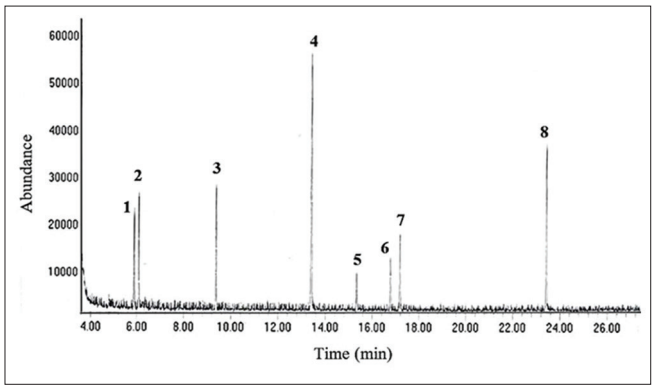

Figure 2: Gas chromatography chromatogram of cinnamon oil. The identified components were (1) 3-hexanol, (2) 2-hexanol, (3) linalool, (4) cinnamaldehyde, (5) eugenol, (6) caryophyllene, (7) cinnamyl acetate, and (8) benzyl benzoate

and the physical appearance of their finished products are shown in Table 3. Among the five formulations, F4 met our specific requirements and was selected for stability testing. The freshly prepared F4 was water in silicone oil emulsion with fairly high viscosity and a slightly acidic pH. The color was the lightest yellowish-brown, with mild and pleasant odor of light cinnamon. The product gave a good texture without separation, providing good coverage and spreadability.

Stability testing

After storage at room temperature for 1 month or 6 cycles of the heating/cooling cycle, F4 still had a good texture, without phase separation. The viscosity, color, scent, and pH did not change, and microbial growth was not observed. The percentage of remaining cinnamaldehyde in F4 was over 90%. These findings indicate that F4 was physically, chemically, and microbially stable under storage conditions and may remain stable throughout the intended shelf-life period. Evaluation of the stability of F4 concealer is shown in Table 4.

F4 showed anti-*P. acnes* activity with the inhibition zones of 18.00 ± 1.41 mm. This was less than that observed from the pure cinnamon oil, which may be due to the restricted spreadability of cinnamon oil through a viscous product. Base formulations showed no inhibitory effect. Anti-*P.*

Table 3: The ingredients of concealer F1-F5 and the physical appearance of F4

Ingredients	% w/w				
	F1	F2	F3	F4	F5
Cinnamon oil	0.5	0.5	0.5	0.5	0.5
Kojic dipalmitate	2.0	2.0	2.0	2.0	2.0
Dimethicone	12.0	12.0	12.0	12.0	12.0
Cyclopentasiloxane (and) PEG/PPG-18/18 dimethicone	15.0	15.0	12.0	17.0	20.0
C30-45 alkyl dimethicone	5.0	5.0	5.0	5.0	5.0
Mineral oil	5.0	5.0	5.0	5.0	5.0
Candelila wax	2.0	1.0	1.0	1.0	1.0
Beeswax	1.0	1.0	1.0	1.0	1.0
Propylene glycol	5.0	5.0	5.0	5.0	5.0
Magnesium aluminum silicate	0.1	0.1	0.1	0.1	0.1
Distilled water	38	39	42	37	34
Tetrasodium ethylenediaminetetraacetic acid	0.1	0.1	0.1	0.1	0.1
Phenoxyethanol	0.5	0.5	0.5	0.5	0.5
Titanium dioxide	10.0	10.0	10.0	10.0	10.0
Bentonite	2.0	2.0	2.0	2.0	2.0
Yellow oxide	1.5	1.5	1.5	1.5	1.5
Red oxide	0.3	0.3	0.3	0.3	0.3
Black oxide	0.1	0.1	0.1	0.1	0.1
	Hard texture	Low viscous / Water separation	Meet specific requirements	Poor spreadability	

Table 4: Evaluation of the stability of F4

Parameter	Freshly prepared	Heating/cooling cycle	Room temperature
Viscosity (cP)	771.3±45.8	836.8±32.5	840.6±28.0
Color	Lightest yellowish-brown	Lightest yellowish-brown	Lightest yellowish-brown
Odor	Mild, pleasant	Mild, pleasant	Mild, pleasant
Separation	No	No	No
Spread ability and coverage	Good	Good	Good
pH	5.72±0.03	5.75±0.04	5.77±0.03
Cinnamaldehyde content (%)	98.05±3.42	96.05±4.60*	97.44±3.28*
Microbial growth	No	No	No

*Percentage remaining content $= \left(\frac{\text{Analyzed amount of cinnamaldehyde at determined time}}{\text{Analyzed amount of cinnamaldehyde at initial time}}\right) \times 100$

acnes activity of F4 stored under room temperature and accelerated conditions was not significantly different, with inhibition zones of 17.25 ± 1.06 mm and 16.75 ± 0.35 mm, respectively. However, instability of trans-cinnamaldehyde after air exposure has been reported, with reactive unsaturated aldehyde being readily oxidized to cinnamic acid before volatile loss;[20] to avoid instability due to oxidation reactions during its use, encapsulation technique and effective antioxidants might be applied.

CONCLUSIONS

Cinnamon oil, galangal oil, eucalyptus oil, and combinations of galangal and cinnamon oil exhibited different extents of anti-*P. acnes* activity. Among all the tested oils, the most effective compound was cinnamon bark oil. It contains cinnamaldehyde as a major constituent, which might be responsible for the anti-*P. acnes* activity. Cinnamon oil might be a promising cosmetic ingredient for anti-acne products. F4, a silicone-based emulsion concealer containing

0.5% w/w cinnamon oil, exhibited physical, chemical, and microbial stabilities after storage in multiple predetermined conditions. F4 concealer could be used to help cover skin imperfections with anti-acne activity. However, further investigation in clinical research is required.

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Conflicts of interest

There are no conflicts of interest.

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