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Formulation of herbal concealer containing fennel seed oil and lavender oil with anti-*Propionibacterium* acnes activity

Wanaree Rukpracha[1], Pattana Sripalakit[2,3] and Aurasorn Saraphanchotiwitthaya[2,4,*]

[1]Cosmetic and Natural Products Research Center, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok,Thailand
[2]Pharmaceutical Biotechnology Research Unit, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand
[3]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand
[4]Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand
[*]Corresponding author: aurasorns@nu.ac.th

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Abstract

Acne is one of the most prevalent dermatologic diseases worldwide. Several substances derived from the herb are currently utilized to treat acne. Interestingly, incorporating essential oils with anti-*Propionibacterium (P) acnes* activity into concealer may help manage acne as well as conceal skin flaws. The study aimed to formulate a concealer with anti-acne properties, using Thai herbal oil with anti-*P. acnes* activities, and determine the formulation's stability. The anti-microbial activity against *P. acnes* DMST 14916 of fennel seed oil (FSO) and lavender oil (LO), and mixtures of the two was evaluated using agar disc diffusion and microbroth dilution assays. Concealers containing oils that have effective anti-*P. acnes* activity were formulated and tested for stability under the determined storage conditions. The result showed that FSO and LO in the ratio of 50:50 exhibited the highest inhibition against *P. acnes* of 23.39±0.48 mm of the inhibition zone with a minimum inhibitory concentration value of 1.56 mg/L. A silicone-based concealer incorporating 0.2% of these blended oils was formulated. F7 provided good texture, spreadability and coverage properties among the seven formulations, and had a pleasant odour. This formulation was biologically, chemically, and physically stable under the determined stability testing storage. In conclusion, a silicone-based anti-acne concealer containing blended oils of FSO and LO (50:50) was successfully developed. This formulation (F7) may be utilized both to manage acne and conceal skin imperfections problems using the benefits of herbal preparations.

Keywords: Anti-acne concealer, Emulsion, Fennel seed oil, Formulation, Lavender oil, Stability testing

1. Introduction

Acne vulgaris is a chronic inflammatory disorder of skin condition, mainly influences teenagers during puberty. It may produce harmful psychological and physical impacts leading to anxiety, depression, low self-esteem and suicidal ideation. Additionally, acne may lead to permanent scarring that is not easy to treat [1,2].

Acne can be classified as noninflammatory (closed or open comedones) and inflammatory lesions (nodules, pustules, and papules) [3]. The pathogenesis of these lesions are multifactorial processes involving high sebum levels, bacterial infection, and hyperkeratinisation coupled with hormonal levels [4]. *Propionibacterium acnes* is a Gram-positive bacterium that plays a crucial role in acne pathogenesis. This bacterium secretes an extracellular lipase that hydrolyses sebum triglycerides to form glycerol and free fatty acids and induces certain inflammatory mediators. These may contribute to the formation of comedones and cause leucocyte attraction to the follicle, inducing inflammatory skin conditions. While hair follicles become clogged with dead skin cells, they are broken

down and secret sebum, dead cells together with bacteria onto the nearby skin, resulting in a spectrum of acne severity [5].

Acne with mild to moderate inflammation can be generally treated with topical antibiotics, anti-inflammatory drugs and comedolytic agents, while modest to severe acne should be treated using systemic therapies, including oral antibiotics, isotretinoin and hormones [6,7]. Conversely, these treatments may cause unwanted side effects, and the prolonged use of antibiotics to treat acne vulgaris may increase antibiotic resistance to acne-causing bacteria [6]. As such, the advancement of low-cost, secure, and effective anti-acne medicines is a necessary challenge to overcome these limitations. Herbal medicines are a significant resource for the production of lead compounds of anti-acne drugs [8]. Moreover, natural compounds derived from herbs are currently used for acne treatments. Several herbal medicines have been reported for their safety or reduced adverse effects, leading to the gradual development of interest in the use of medicinal plants in recent years.

Fennel seed essential oil (FSO) has been reported to have antibacterial properties against acne-causing bacteria [9]. However, the application was limited due to its spicy odour. Lavender oil (LO) is one of the best-known and most useful essential oils for aromatherapy. It has been reported for antibacterial properties and contained antioxidants that help nourish the skin, heal dry skin, and reduce dark circles and acne scars [10]. Literature has also proved that the combination of medicinal plants produces a synergistic therapeutic effect with improved patient compliance [11]. Therefore, the combination of FSO and LO was chosen for their anti-*P. acnes* effects in anti-acne concealers and LO was used for beneficial masking of the spicy odour of the FSO. This research aimed to investigate the antimicrobial activity against *P. acnes* of fennel seed oil (FSO), lavender oil (LO), and mixtures of the two, in comparison with tea tree oil and clindamycin. A silicone-type concealer incorporated with the effective oil was formulated. The formulation which attained the requirement was selected for stability testing.

2. Materials and methods

2.1 Chemicals

This research was approved by the Institutional Biosafety Committee, Naresuan University, Thailand. *Propionibacterium acnes* DMST 14916 was acquired from the National Institutes of Health, Thailand. FSO, LO and tea tree oil were bought from Chemipan Corporation Company, Thailand. Other formulation ingredients were obtained from Namsiang Trading Company, Thailand and Phitsanu Chemical Company, Thailand. Clindamycin was purchased from R.P.C. International Company, Thailand.

2.2 Microbial cultivation

P. acnes was anaerobically grown on BHI agar at 37°C for 48 h. Consequently, *P. acnes* cells were dispersed in BHI broth and adjusted to an average optical density of 0.90 (595 nm), which is equivalent to the 0.5 McFarland turbidity standard.

2.3 Disc diffusion assay

The antibacterial activity of FSO, LO, and mixtures of FSO and LO at 70:30, 50:50, and 30:70 was determined using the agar disc diffusion method. A *P. acnes* suspension of 300 µL (1.5×10^{11} CFU/L) was spread onto BHI agar and left for 15 min. Twenty microliters of tested samples were soaked into 6.0 mm diameter paper discs and these were put directly onto the BHI agar and gently pressed. The plates were kept under anaerobically incubation for 48 h at 37°C. Thereafter, the measurement of the inhibition zone was done using a vernier calliper [12]. Clindamycin (0.2%) was utilized as a positive control, whilst dimethyl sulfoxide (DMSO) in distilled water (0.1%) was a negative control. The anti-*P. acnes* activity of the concealer was determined by applying 0.5 g of the concealer to the agar well instead of using the filter paper disc. This was an appropriate amount of concealer for fulfilling in the agar well and could be applied to conceal the affected face area.

2.4 Broth dilution assay

The broth dilution assay was employed to quantitatively measure the antimicrobial activities of the tested essential oils against *P. acnes*. Various concentrations of the tested essential oils were prepared by 2-fold dilution in BHI broth using DMSO (0.1%) as a solubilizing agent. One millilitre of *P. acnes* (1.5×10^{11} CFU/L) suspension in culture broth and 1 mL of the tested oils were added to the test tube, mixed well before adding liquid paraffin and sealing firmly with parafilm. Each tube was incubated anaerobically for 24 h at 37°C. The lowest dose of tested samples that gave a clear mixture, indicating the inhibition of bacterial growth, was defined as the MIC (minimum inhibitory concentration) value [12]. BHI broth containing DMSO (0.1%) was used as a negative control and clindamycin (0.2%) was used as a positive control.

2.5 GC-MS analysis

GC was used for chemical analysis (Hewlett-Packard Company, USA), furnished with an HP-5MS column (30 m × 0.25 mm i.d. × 0.25 μm). The column temperature was programmed to rise at a rate of 5°C per min from 70-280°C. The carrier gas was helium at the flow rate of 1.0 mL/min. The scan range of 50-500 amu was used [13]. Trans-anethole, which is the major ingredient in FSO, α-terpinyl acetate in LO and limonene in both essential oils were designated as markers. They were diagnosed by matching the retention time of the peaks at 14.07, 15.73 and 6.90 min, respectively. To evaluate their content in the formulation, 5 mL hexane was added to 0.5 g concealer and mixed thoroughly. After 15 min of sonication, the mixture was filtered through a 0.45-micron filter and the collected filtrate was tested by GC-MS analysis.

2.6 Concealer formulation

Our concealer was required to be stable water in silicone emulsion type for yellow skin tones with anti-*P. acnes* activity provided good coverage, viscous texture, and spreadability. Seven concealer formulations were prepared to contain FSO and LO, labelled F1-F7, with optimization of the composition of emulsifiers, dimethicone, wax, propylene glycol, pigments and distilled water. The silicone phase, which included various amounts of dimethicone, and the oil phase, which included wax and Span 60®, were heated to 75°C and then mixed by homogenizer at 3000 rpm. Veegum® was dispersed in distilled water, mixed with propylene glycol, Tween 60, tetrasodium EDTA and phenoxyethanol, and heated to 70°C. The water phase was added continuously to the mixture of dimethicone and oils and mixed until an emulsion was achieved. Titanium dioxide was consistently incorporated with pigments and then mixed well with the emulsion. When the preparation had cooled down, FSO and LO were mixed by a homogenizer at 3000 rpm. The formula that qualified for our determined requirements was chosen, and the physical, chemical and microbiological stability was evaluated. The emulsion type of the freshly prepared formulation was determined by staining an emulsion with water-soluble dye (0.5% methylene blue dye solution) and observing the results under the microscope.

2.7 Stability testing

The selected concealer formulation that met the specific requirements was stored at room temperature (25±2°C) for one month. The accelerated stability was conducted by heating/cooling for 6 cycles between 4°C and 45°C, each of 24 h. After 30 days, we determined the physical stability, including separation, spreadability, colour, odour, and coverage properties through observation. The measurement of pH was done using a pH meter (Mettler-Toledo International Inc., USA). The viscosity change was determined using a Brookfield DV-III viscometer (AMETEK, USA). The trans-anethole, α-terpinyl acetate and limonene content was analysed using GC-MS for chemical stability. The spread plate technique on brain-heart infusion (BHI) agar was used to test microbial contamination (total viable count) to determine microbiological stability. Anti-*P. acnes* activity of the selected concealer was evaluated.

2.8 Statistical analysis

Experiments were done in triplicate. Statistical analysis was conducted using a one-way analysis of variance and reported as mean ± standard deviation (SD). A p-value of less than 0.05 indicates statistically significant.

3. Results and discussion

3.1 Antimicrobial activity of fennel seed oil (FSO) and lavender oil (LO) against P. acnes

Numerous natural compounds including microbial and medicinal plants derived products have been reviewed for their anti-acne property. In this study, we used tea tree oil and clindamycin as the herbal and medicinal positive control, respectively. Tea tree oil is a monoterpene-rich essential oil extracted from *Melaleuca alternifolia*. It has antimicrobial and anti-inflammatory activity [14] and is widely utilized in acne care products. Clindamycin is a semi-synthetic antibiotic that belongs to the class of lincosamide. Clindamycin works by blocking protein synthesis in both anaerobic and aerobic bacteria, such as *P. acnes* and *S. aureus*. [15]

The anti-*P. acnes* activity of the essential oils of fennel seed (*Foeniculum vulgare*) and lavender (*Lavandula angustifolia*) were assayed in this study. Previous studies have revealed that FSO presents various biological activities, including antimicrobial, anti-inflammatory, antioxidant, anti-cancer, anti-spasmodic, anti-hypertensive and hepatoprotective properties. The major constituents in FSO were trans-anethole, limonene, fenchone and methyl chavicol [16,17]. Trans-anethole has been widely used in the food, cosmetic and healthcare industries, and many reports prove its antimicrobial, insecticidal, larvicidal and antioxidative properties [18]. LO has also been

shown to possess various biological properties, such as anti-inflammatory, analgesic, anxiolytic and antimicrobial benefits [19]. There is a substantial difference in the chemical constituents of LO, due to distinct plant cultivation areas, genotypes and extraction methods. However, linalool, linalyl acetate and terpinyl acetate comprise the highest quantity of bioactive components detected in LO, and their anti-microbial activity has been reported [19,20]. Moreover, a previous study showed that limonene, which has been found in various essential oils, including FSO and LO, had antimicrobial activity against *P. acnes* [21].

As shown in Table 1, the anti-*P. acnes* activity of FSO and LO, determined by agar disc diffusion assay are performed. The clear zones have been observed when assaying by FSO, LO and their combination, showing that they were capable of suppressing or decelerating *P. acnes* growth. However, these clear zones were less than those from tea tree oil and clindamycin solution. The highest inhibition zone area was detected with FSO:LO at a ratio of 50:50 which was significantly higher than that from FSO alone, followed by 30:70 and 70:30, respectively. These results revealed that the mixing of FSO with LO can increase the clear zone, supporting anti-*P. acne* activity. Therefore, FSO:LO at a ratio of 50:50 was selected for further study.

As illustrated in Table 2, the lowest concentration of essential oils and clindamycin solution (positive control) that led to the nonappearance of visually observed growth of *P. acnes* was determined. The MIC value of LO against *P. acnes* was 6.25 mg/L, while the MIC value of FSO and the mixture of both essential oils (50:50) was 4-fold more potent than LO (1.56 mg/L). Previously, Zu, et al [22] reported that, among several essential oils, LO at a concentration of 0.25% exhibited the strongest bactericidal activities, and *P. acnes* was entirely killed after 5 min.

Moreover, Orchard, et al [23] reviewed the antimicrobial activities of FSO against *P. acnes* and *Staphylococcus epidermidis*, showing MIC values of 0.50 and 2.00 g/L, respectively. Our results revealed the antimicrobial activity of FSO and LO against *P. acnes*, which was consistent with previous research, leading to the possible use of both essential oils in the treatment of acne. Based on these findings, a mixture of FSO:LO (50:50) at a concentration of 0.2% was incorporated into the formulation. It was found that blending with LO benefited by masking the spicy odour of the FSO. Moreover, using mixed oil may reduce the risk of skin irritation and allergy compared with a high dose of single essential oil.

Table 1 Antimicrobial activity of tested essential oils and the F7 formulation against *P. acnes* by agar disc diffusion method.

Tested sample	Diameter of inhibition zone (mm)
Fennel seed oil (100%)	18.78±0.79*
Lavender oil (100%)	21.72±0.77*
Fennel seed oil : Lavender oil (70:30)	20.50±1.36*
Fennel seed oil : Lavender oil (50:50)	23.39±0.48*,**
Fennel seed oil : Lavender oil (30:70)	21.06±1.00*
Tea tree oil (100%)	37.05±5.42
Clindamycin solution (0.2%)	34.26±0.56
DMSO (0.1%; Blank)	0.00±0.00
F7 concealer (Freshly prepared)	15.93±1.43*,***
F7 concealer (storage at 1 month)	13.50±0.78
F7 concealer (heat cool cycling)	14.20±2.43*

Data represented mean ± SD of triplicate experiments. *,** p <0.05 when compared with clindamycin solution and fennel seed oil, respectively, and*** p <0.05 when compared with Fennel seed oil : Lavender oil (50:50).

Table 2 Minimum inhibitory concentration (MIC) of tested essential oils against *P. acnes* by broth dilution assay.

Tested sample	MIC (mg/L)
Fennel seed oil (100%)	1.56
Lavender oil (100%)	6.25
Fennel seed oil : Lavender oil (50:50)	1.56
Clindamycin solution (1.0%)	0.39
DMSO (0.1%; Blank)	-

3.2 Formulation of concealer

The composition of concealer F7 is shown in Table 3. Cyclopentasiloxane (and) PEG/PPG-18/18 dimethicone, Span 60® and Tween 60 were used as emulsifiers. Beeswax, carnauba wax and cetyl alcohol were used as stiffening agents. Veegum® and bentonite were utilized as thickening agents. Two silicon-based polymers, dimethicone and cyclodimethicone, were used in enhancing the evenness of the skin. Blended oils of FSO and LO (50:50) were incorporated in the formulation as anti-acne agents, together with other inactive ingredients.

Amongst the seven formulations, F7 was selected for stability testing as it gave a good texture and provided better coverage and spreadability (Figure 1), without separation. Observation of the staining F7 with methylene blue under a microscope showed the blue drop of water distributed thoroughly in the sample. It could be assumed that F7 was a water-in-silicone oil emulsion type. The texture was relatively high in thickness with neutral pH. It

was the lightest yellowish-brown in colour and had a pleasing odour with a mild spicy aroma. The anti-*P. acne* activity of the F7 concealer was assayed by the agar disc diffusion method. A reduction of *P. acnes* was observed around the application area, indicating the anti- *P. acnes* activity of the F7 concealer (Table 1). Moreover, the inhibition zone by the F7 concealer when freshly prepared, stored for 1 month and after heat-cool cycling was not statistically different.

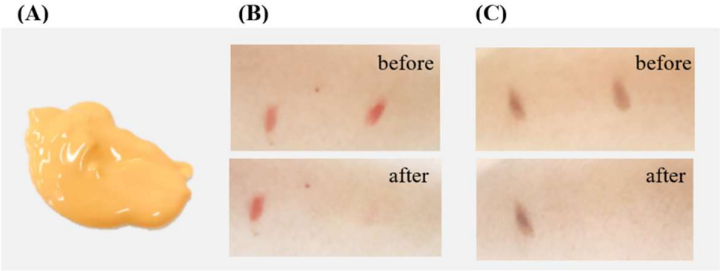

Figure 1 (A) Physical appearance of F7 concealer and its coverage property on (B) red and (C) dark spots.

Table 3 The concealer ingredients of formula F7.

Ingredients	Content (wt.%)
Dimethicone	18.0
Cyclodimethicone	16.0
Cyclopentasiloxane (and) PEG/PPG-18/18 dimethicone	10.0
Span 60®	3.0
Cetyl alcohol	1.0
Carnauba wax	1.0
Beeswax	0.5
Propylene glycol	4.0
Tween 60	2.0
Bentonite	0.4
Veegum®	0.4
Water	29.0
Tetrasodium EDTA	0.1
Phenoxyethanol	0.5
Titanium dioxide	12.0
Yellow oxide	1.6
Red oxide	0.16
Black oxide	0.04
Fennel seed oil	0.1
Lavender oil	0.1

3.3 Stability testing

As shown in Table 4, the stability testing of the F7 concealer was evaluated. At the end of the storage period for 30-days at room temperature or under cooling/heating conditions at six cycles, there was no noticeable change in the product's colour, scent, and pH. However, a slight decrease in viscosity of the formulation kept for 1 month and under cooling/heating conditions was observed. Moreover, F7 still had a good texture, no phase separation, and without microbial growth (data not shown). GC-MS analysis of the blended oils of FSO and LO in the F7 formulation was undertaken. For freshly prepared products, the major components detected in the F7 formulation were trans-anethole (14.07 min), α-terpinyl acetate (15.72 min) and limonene (9.38 min), respectively.

$$\%\text{Remaining content} = \left(\frac{\text{analyzed amount at a determined time}}{\text{analyzed amount at an initial time}}\right) \times 100 \quad (1)$$

This was in accordance with the results of the single oils, where trans-anethole was the major compound in FSO, α-terpinyl acetate was mainly detected in LO, and limonene was found in both FSO and LO, respectively [13,24,25]. These compounds are possibly accountable for the anti-*P. acnes* activity [18-20]. When compared with the formulation stored at room temperature for 30 days and the formulation stored in the heating/cooling condition, the percentage of trans-anethole, α-terpinyl acetate and limonene remaining in F7 was over 90%. Stability testing is usually undertaken according to the established period to confirm the shelf-life of products during the proposed testing period under storage conditions. Real-time testing is generally carried out for less than 12 months while accelerated testing is done for less than 6 months by most authorities. In this study, the finished product has been kept for 30 days and stored under heating-cooling stress conditions within the range of general storage time of stability testing. However, stability testing under a longer duration should be confirmed, allowing significant product degradation under recommended storage conditions closer to the actual stability.

Table 4 Stability assessment of F7 formulation.

Characteristics	Freshly prepared formulation	Formulation kept under storage condition	
		Heating and cooling cycle	Room temperature, 1 month
Viscosity (cP)	621.1±26.25	739.13±26.59*	748.43±36.94*
Colour	light yellowish-brown	light yellowish-brown	light yellowish-brown
Odour	pleasant	pleasant	pleasant
Separation	no	no	no
Spreadability and coverage	good	good	good
pH	6.84±0.03	6.85±0.05	6.83±0.03
Limonene content (%)[a]	98.50±3.40	97.30±3.44	96.58±4.35
Trans-anethole content (%)[a]	99.02±2.10	96.05±4.06	98.54±3.40
α-terpinyl acetate content (%)[a]	97.14±4.36	98.41±3.74	97.44±3.45
Microbial growth	no	no	no

[a]%Remaining content, data represented mean ± SD of triplicate experiments, and $^{*}p < 0.05$ when compared with the freshly prepared formulation.

4. Conclusion

The mixture of FSO and LO at a ratio of 50:50, which gave the maximum inhibition zone by the disc diffusion assay, was evaluated for MIC and incorporated into the formulation. The best formulation was F7, which was a concealer in a silicone-based emulsion type that contained 0.2% of FSO and LO mixture. Under the determined storage conditions, this formulation was biologically, chemically, and physically stable and remained stable during the intended shelf-life time. The F7 formulation could be used to assist conceal skin flaws, as well as provide anti-acne activity. However, clinical studies need to be undertaken to investigate further.

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