science, engineering and health studies

Research Article

Science, Engineering and Health Studies
https://lio1.tci-thaijo.org/index.php/sehs
ISSN (Online): 2630-0087

Monte Carlo dosing simulations of topical terpinen-4-ol from *Zingiber cassumunar* oil against *Cutibacterium acnes* and *Staphylococcus aureus*

Ei Ei Win[1], Kotchaphan Chooluck[2], and Wichit Nosoongnoen[1]*

[1] Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand
[2] Department of Manufacturing Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand

***Corresponding author**: Wichit Nosoongnoen
wichit.nos@mahidol.ac.th

***Received**: 26 April 2024*
***Revised**: 9 October 2024*
***Accepted**: 13 October 2024*
***Published**: 31 December 2025*

***Citation**: Win, E. E., Chooluck, K., & Nosoongnoen, W. (2025). Monte Carlo dosing simulations of topical terpinen-4-ol from Zingiber cassumunar oil against Cutibacterium acnes and Staphylococcus aureus. Science, Engineering and Health Studies, 19, 25050021.*

ABSTRACT

Plai oil is an essential oil derived from the steam distillation of *Zingiber cassumunar* rhizome. The oil contains terpinen-4-ol (T4) as its major component. This study developed and validated gas chromatographic–mass spectrometric methods for quantifying and investigating the physicochemical properties of T4 and determined the effective dosage regimens of T4 against *Cutibacterium acnes* and *Staphylococcus aureus* using Monte Carlo simulation. The results demonstrated that the aqueous solubility of pure T4 was higher than that of T4 in plai oil, and the log K_{ow} of T4 in the pure form and plai oil were in the ranges of 0.73–2.83 and 0.77–2.88, respectively. The simulation analysis suggested topical pure T4 dosage regimens of 1.50 and 2.00 g/cm^2 every 12 h to suppress *C. acnes* (MIC 1,489.60 µg/mL) and *S. aureus* (MIC 2,327.50 µg/mL), respectively, to achieve the PKPD targets of C_{max} > MIC and AUC > 2×MIC. When converted to plai oil (21.23% w/w of T4), the topical dosage regimens were 7.07 and 9.42 g/cm^2 every 12 h, respectively. At higher MIC or PKPD targets, the topical dosage regimens of T4 and plai oil were 2.70 and 12.72 g/cm^2 for *C. acnes* and 3.00 and 14.13 g/cm^2 for *S. aureus*, every 12 h.

Keywords: *Cutibacterium acnes*; Monte Carlo simulation; PKPD; plai oil; *Staphylococcus aureus*; Terpinen-4-ol; *Zingiber cassumunar*

1. INTRODUCTION

Acne is a prevalent skin issue in adolescents. The condition is attributed to various factors, including excessive sebum production, occlusion of skin pores due to hyperkeratosis, infections caused by bacteria such as *Cutibacterium acnes*, and inflammation (Hazarika, 2021). Of particular significance, excessive activation of *C. acnes* emerged as the primary catalyst for acne development and the subsequent severe inflammatory reactions (Kirschbaum & Kligman, 1963).

Antibiotics have been the primary therapeutic approach for acne formation induced by *C. acnes* and other bacteria such as *Staphylococcus aureus*. Nonetheless, the escalating issue of antibiotic resistance, emerging from excessive and inappropriate use, has posed a significant threat to global public health (Walsh et al., 2016; Karadag et al., 2021; Legiawati et al., 2023). In this regard, numerous researchers have explored the potential bioactivities and therapeutic applications of natural herbs, evaluating active compounds possessing antibiotic and anti-inflammatory

properties to develop alternative treatments that could overcome the challenges posed by antibiotic resistance (Nurzyńska-Wierdak et al., 2023).

Noteworthy essential oils, like Australian tea tree oil sourced from *Melaleuca alternifolia* L. and plai oil from *Zingiber cassumunar* Roxb, have been found to possess significant antibacterial and antifungal properties, primarily due to the presence of their active component, terpinen-4-ol (T4) (Carson et al., 2006; Pithayanukul et al., 2007; Sharifi-Rad et al., 2017; Han et al., 2021; Yasin et al., 2021). *Zingiber cassumunar* Roxb, a medicinal plant extensively cultivated in tropical Asia, produces plai oil via steam distillation of its rhizome, which is used for pain relief and as anti-inflammatory and antibacterial agents. Plai oil is found to be active against many gram-negative and gram-positive bacteria including *C. acnes*, *Escherichia coli*, *Staphylococcus epidermidis*, *and S. aureus.* The minimum bactericidal concentration (MBC) of plai oil was found in the range of 0.62%–2.5% v/v (Pithayanukul et al., 2007; Chongmelaxme et al., 2017; Alshiekheid et al., 2022). A study from Indonesia revealed that 10% v/v *Zingiber purpureum* (another synonym of *Z. cassumunar*) inhibited gram-positive and gram-negative bacteria, particularly those resistant to a variety of antibiotics, including beta-lactams, carbapenems, and first-, second-, and third-generation cephalosporins (Tandirogang et al., 2022).

The T4 concentration in tea tree oil consistently remains above 40%, typically ranging from 41% to 42.35% (Hart et al., 2000; Mondello et al., 2022). T4 is also found in plai oil at a concentration of 32%–48.1% (Pithayanukul et al., 2007; Chooluck et al., 2012). It is extensively used in cosmetic and pharmaceutical products due to its favorable permeability and pharmacological effects, including antibacterial, antifungal, antiviral, anti-inflammatory, and analgesic properties (Dong et al., 2015). The content of T4 in plai oil may slightly change based on the methods used to extract the oil from the plant (Sukatta et al., 2009; Singsai et al., 2022). A previous study reported that the T4 content in plai oil obtained using hexane extraction was 33.11%–49.36%, while that obtained through hydro-distillation ranged from 21.85%–29.96% (Sukatta et al., 2009). Although the minimum inhibitory concentration (MIC) of T4 against bacteria in plai oil has not been specifically reported, numerous studies have reported the MIC of T4 extracted from tea tree oil. The MIC of T4 against *S. aureus* varies between 0.25% and 2.5% v/v (Cordeiro et al., 2020; Johansen et al., 2022). Moreover, the MIC against *S. aureus* and *C. acnes* in the T4 group was in the range of 0.16%–0.31% v/v (Raman et al., 1995). Given that the percentage of T4 in tea tree oil and plai oil falls within a similar range, it is reasonable to assume that the MIC values of T4 extracted from tea tree oil would also be applicable to plai oil.

Chooluck et al. (2012) conducted cutaneous microdialysis in rats to investigate the dermal pharmacokinetics of T4 after topical application of *Zingiber cassumunar* (plai) oil (Chooluck et al., 2012). However, there is a lack of research on the appropriate dosage regimens of T4 and plai oil for treating skin infections caused by *C. acnes* and/or *S. aureus* in humans.

Monte Carlo simulation (MCS) is a statistical method for predicting and evaluating antimicrobial drug effectiveness using antimicrobial pharmacokinetic parameters, the MIC of the microorganism, and the pharmacokinetic/pharmacodynamic (PKPD) index (Vinks et al., 2014; Roberts et al., 2011). MCS is a beneficial tool for clinicians and researchers to develop optimal empirical therapeutic regimens (Asín-Prieto et al., 2015). Because there are no individual therapeutic drug monitoring data for T4, a pharmacokinetic and pharmacodynamic study using MCS is better suited to determine the best dose regimens for specific MICs.

Although MCS cannot predict the concentration-time profile for a new patient, it can estimate the expected range of the concentration-time profile for a selected population under a specific dosing regimen. To describe the distribution of the expected antibiotic concentration profile for a chosen dosing regimen, several virtual populations (typically 5,000–10,000 subjects) must be simulated. The simulation results of the virtual population can provide a forecast of the probability of target attainment (PTA), which represents the probability of achieving a given value of the PK/PD index associated with antibiotic efficacy at a given MIC.

This study aimed to develop and validate gas chromatographic–mass spectrometric (GC-MS) methods for quantifying and investigating the physicochemical properties of T4. Furthermore, the effective dosage regimens of T4 against *C. acnes* and *S. aureus* were assessed using the concept of the PK/PD of T4, the MCS process, and the MIC of bacteria.

2. MATERIALS AND METHODS

2.1 Materials

Reference standards for (+)-terpinen-4-ol (≥98.5%) and methyl salicylate (≥99.5%) were purchased from Sigma–Aldrich (Buchs, Switzerland). GC-grade hexane and octanol were supplied by Thermo Fisher Scientific (Loughborough, UK) and Sigma–Aldrich (Steinheim, Germany). Plai oil was obtained from Kovic Kate International Co., Ltd (Batch no. PE-13222-01, Bangkok, Thailand). Deionized water was obtained from Thai Nakorn Patana (Nonthaburi, Thailand).

2.2 Instrumentation and GC-MS conditions

The analysis was carried out using a gas chromatography (GC) system coupled with a quadrupole mass spectrometer (GCMS-QP2010, Shimadzu, Kyoto, Japan). The GC-MS conditions were modified slightly from those in our previous study (Chooluck et al., 2013). The compounds were separated on a BPX5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness, SGE Analytical Science, Ringwood, Australia). Ultra-high-purity helium (99.999%) was employed as the carrier gas at a flow rate of 1.0 mL/min. Sample injection (1 µL) was performed in splitless mode. The initial oven temperature was set at 45°C with a holding time of 1 min. It was then increased by 10°C/min to 115°C, where it was maintained for 5 min, followed by an increase to 200°C at a rate of 80°C/min, with a holding time of 4 min. The temperatures of the injection port, interface, and ion source were set to 230°C, 200°C, and 200°C, respectively. The T4 and IS peaks in plai oil were identified by comparing the retention times and ion fragmentation with those of the pure standards. Other compounds were identified via a similarity search of the WILEY7 mass spectral library. Electron impact ionization was performed at an ionization energy of 70 eV. To quantify T4 in the plai oil, MS was operated in SCAN mode (*m/z* 30–400). To investigate the physicochemical

properties of T4, a quantitative method using the selected-ion monitoring (SIM) mode was also developed and validated to minimize interference from the matrix samples and achieve better sensitivity. The programmed column temperature was as follows: 60°C for 1 min, then 60°C–115°C at 10°C/min and held for 5 min, and 115°C–200°C at 80°C/min and held for 4 min. The monitored ions were as follows: for T4, *m/z* 71, 93, and 111, and for methyl salicylate, *m/z* 92, 120, and 152. The area ratio of 71/120 was used for quantification, whereas the other ions were used as qualifiers. GC-MS solution software (version 2.50) was used for gas chromatographic and mass spectral analyses.

2.3 Standard and sample preparations

The standard stock solutions of T4 and methyl salicylate, used as the internal standard (IS), were prepared in hexane at concentrations of 2 and 10 µg/mL, respectively. The stock solutions were stored at 20°C for a maximum of 1 month. For the recovery study in deionized water and Ringer's solution, a standard stock solution of T4 was freshly prepared in deionized water.

For the GC-MS operated in SCAN mode, an accurate weight of the plai oil was transferred into a volumetric flask and diluted with hexane to obtain a final oil concentration of 1.75 µg/mL, spiked with the IS working solution to obtain a concentration of 1.2 µg/mL. For the developed SIM method, oil and IS were diluted and spiked to obtain concentrations of 140 and 360 ng/mL, respectively.

2.4 Method validation

The method was validated according to the AOAC guideline on single-laboratory validation of chemical methods for dietary supplements and botanicals (AOAC, 2002). The quantitative assay of T4 in plai oil using MS operated in SCAN mode was validated by evaluating the selectivity, linearity, intra- and inter-day accuracy, and precision. The method selectivity was verified by comparing the crude oil chromatograms with those of the blank solvent and standard solutions. The calibration standards were prepared by diluting the stock solution with hexane to obtain final T4 concentrations of 0.35, 0.70, 1.00, 1.40, and 1.80 µg/mL. An appropriate amount of the IS working solution was transferred to each calibration standard to obtain a final concentration of 1.2 µg/mL. The linearity was investigated by analyzing the standard solutions of T4 at five concentrations. Calibration curves were constructed by plotting the nominal standard T4 concentration (X-axis) and peak area ratios of T4 to IS (Y-axis). The limits of detection (LOD) and quantification (LOQ) were estimated at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of diluted solutions with known concentrations. The intra-day and inter-day precision values were evaluated at three concentration levels of T4 (0.70, 1.00, and 1.40 µg/mL) by analyzing standard solutions on the same day (n = 3 for each concentration level) and on three different days (n = 9), respectively. Subsequently, the relative standard deviation was calculated for each concentration. The accuracy was examined by the recovery of known amounts of standards spiked into the plai oil solution (1.75 µg of plai oil/mL) to obtain additional T4 concentrations of 0.18, 0.70, and 1.00 µg/mL. The experiment was performed in triplicate.

To minimize interference from the matrix samples and achieve better sensitivity, the GC-MS was operated in the SIM mode. Linearity was evaluated at 17, 70, 140, 280, 560, and 1,120 ng/mL. The IS working solution was spiked to obtain a concentration of 360 ng/mL. The precision was determined as described above at concentrations of 70, 420, and 850 ng/mL. The recoveries of T4 were determined by spiking a known amount of T4 in the oil solution (143 ng of oil/mL), octanol solution, deionized water, and Ringer's solution at the levels of 70, 420, and 850 ng/mL.

2.5 Aqueous solubility study

The solubility experiments were carried out using the isothermal shake-flask method, which was slightly modified from a previous report (Cal, 2006). An accurately weighed amount of pure T4 and plai oil (21.29% w/w of T4) was separately added to 2.0 mL of deionized water or Ringer's solution, corresponding to T4 contents of 0.2, 0.4, 0.8, and 1.0 g. The mixtures were placed in an incubator shaker (200 rpm) at 25°C ± 1°C for 72 h. After centrifugation (3,000 g, 15 min), the oil layer (upper) was carefully removed using a micropipette. The aqueous phase was extracted with 2 mL of hexane by vortexing for 30 s. The organic phase was analyzed by GC-MS. The experiment was performed in triplicate.

2.6 Partition coefficient study

A partitioning study of T4 in its pure form and plai oil was performed using the shake-flask method, which was slightly modified from a previous report (Vilas-Boas et al., 2022). Octanol and water were pre-saturated with each other at a ratio of 1:1 for 24 h before use in this experiment. Solutions of known T4 concentrations (0.125, 0.5 and 1.0, and 2.0 mg/mL) were prepared in octanol, and a ratio of 1 mL of octanol to 1 mL of water was used in the partitioning study. The resulting two-phase mixture was incubated at 37°C on a shaker at 20 rpm for 12 h to establish equilibrium. Both phases of the samples were analyzed separately using the GC-MS method. The octanol fractions were diluted with hexane before analysis to reduce column overloading by octanol. The partition coefficient is expressed in logarithmic form as K_{ow}, which is the concentration of T4 in octanol divided by that in the aqueous solution.

2.7 Microbiological MIC data

The MIC data for T4 against *C. acnes* and other skin infection bacteria were obtained from a previous study. The MIC values of T4 against those bacteria were 100 µg/mL for *S. gordonii*, 200 µg/mL for *Prevotella intermedia*, 800 µg/mL for *S. pyogenes* (Lee et al., 2013), 0.16% v/v for *C. acnes* (Raman et al., 1995), 0.25% v/v for *S. aureus* (Cordeiro et al., 2020), and an MBC of 1,600 µg/mL (Lee et al., 2013) for *S. pyogenes.* In this study, the unit %v/v was converted into µg/mL before being applied in the MCS process.

Therefore, the conversion of 0.16% and 0.25% v/v to µg/mL is achieved by multiplying the respective %v/v values by the density of T4 and 10,000. The density of T4 (0.931 g/mL at 25°C) serves as the conversion factor. Subsequently, 0.16% v/v corresponds to 1,489.6 µg/mL, and 0.25% v/v is equal to 2,327.5 µg/mL.

2.8 Monte Carlo simulation

2.8.1 PK parameters

The raw data regarding the concentration of T4 following topical application of plai oil, as presented in the supplementary file, were obtained from Chooluck and her colleagues from the Faculty of Pharmacy, Mahidol University, Thailand. Phoenix 64 WinNonlin® version 8.3.4.295 (Certara USA, Inc., Princeton, NJ), licensed to the Faculty of Pharmacy, Mahidol University (February 9, 2023 to February 8, 2025), was used to predict the pharmacokinetic parameters of T4. The area under the concentration-time curve from 0 to the last measured value ($AUC_{0\text{-last}}$) was calculated using the linear trapezoidal rule implemented in the non-compartmental analysis using Phoenix64 WinNonlin® software, as shown in Equation 1 and $AUC_{0-\text{infinity}}$ was calculated using Equation 2. The maximum concentration (C_{max}) and the time to reach the maximum concentration (T_{max}) were determined directly from the concentration vs. time curve. The terminal elimination rate constant (k_e) was calculated from the log-linear portion of the elimination curve using linear regression. The half-life of the drug ($t_{1/2}$), Volume of distribution of the drug (V_d), and clearance of the drug (CL) were calculated using the following Equations 3–5.

$$AUC_{0\text{-last}} = ½ (C_1+C_2) (t_2-t_1) \quad (1)$$

$$AUC_{0\text{-infinity}} = AUC_{0\text{-last}} + AUC_{\text{last-infinity}} \quad (2)$$

$$AUC_{\text{last-infinity}} = C_{last}/k_e,$$

where C_{last} is the last concentration measured.

$$t_{1/2} = \text{In}2/k_e \quad (3)$$

$$CL = \text{Dose}/AUC_{0\text{-infinity}} \quad (4)$$

$$V_d = CL/k_e \quad (5)$$

The k_a value was obtained from a one-compartment analysis of Phoenix64 WinNonlin® simulations with a value of 1.47 h^{-1}. These calculated PK parameters, such as V_d, CL, k_e, and k_a were used as the initial parameters in Oracle Crystal Ball® software (version 11.1.2.4.850) to generate the plasma concentration profile of 10,000 patients using the one-compartment formula in Equation 6.

$$C = \{k_a*F*D/ V*(k_a-k_e)\}*[e^{-ket} - e^{-kat}] \quad (6)$$

2.8.2 Dosage regimens

Because the MIC was quite high and the C_{max} of T4 was very low at an original dosage of 1.0–3.8 mg/cm^2. Therefore, the following dosage regimens (1.0, 1.5, 2.0, 2.2, 2.3, 2.5, 2.7, and 3.0 g/cm^2 every 6, 8, and 12 h) were simulated using the Oracle Crystal Ball® software to cover all the MIC ranges.

2.8.3 PKPD index

In the time-kill study conducted by Johansen et al. (2022) a combination of T4 and α-terpineol in a ratio of 10:1 was utilized at concentrations equivalent to the MIC, two times the MIC (2×MIC), and four times the MIC (4×MIC) to assess bactericidal activity. The results of the time-kill assay indicated that the mixture demonstrated bactericidal effects against *S. aureus* and other bacteria, including methicillin-resistant *S.* (MRSA), within a timeframe of less than 1 h at 4×MIC, resulting in a 3-$\log_{10}$ reduction in the bacterial count (Johansen et al., 2022). Since precise PKPD targets for T4 were not available, three estimated targets of C_{max} ≥ MIC, C_{max} ≥ 2×MIC, and AUC ≥ 2×MIC were employed in the simulation.

Subsequently, 10,000 virtual patients were created for each dosage regimen using Oracle Crystal Ball® software to construct the drug plasma concentration-time profiles until steady state was achieved. Before the simulation of 10,000 trials, all parameters were assumed to follow a log-normal distribution. Subsequently, the percentage probability of target attainment (%PTA) of T4 was computed for each dosing regimen across every MIC range to determine the proportion of subjects attaining the predefined therapeutic targets.

3. RESULTS

3.1 Method validation

An accurate, simple, and reproducible GC-MS method was developed and validated for the determination of T4 in plai oil and its application in solubility and partition coefficient studies. Figure 1 shows representative GC-MS chromatograms of blank hexane, standard solutions, and plai oil. The retention times of T4 and IS were 11.87 and 12.40 min, respectively. The chromatogram shows that the T4 peak was satisfactorily separated from other substances, and no interfering species were present at the retention time of the investigated analytes. The method showed good linearity in the range of 0.35–1.80 µg/mL. The correlation coefficient (r^2) was >0.999 for all validation batches. The LOD and LOQ of this method were 0.03 and 0.09 µg/mL, respectively. The intra-day and inter-day precision values were less than 2.3% and 3.7%, respectively. The average recovery of T4 in plai oil was 103.0% ± 4.2%.

A GC-MS method using the SIM mode was developed and showed good linearity in the range of 17–1120 ng/mL with r^2 >0.999 for all validation batches. The retention times of T4 and IS were 9.33 and 9.84 min, respectively (Figure 2). The LOD and LOQ of this method were 4.3 and 14.5 ng/mL, respectively. The intra-day and inter-day precision values were less than 1.1% and 0.8%, respectively. The mean recovery of T4 in plai oil, octanol, deionized water, and Ringer's solution was 104.7% ± 2.3%, 100.1% ± 0.9%, 100.7% ± 0.9%, and 103.8% ± 1.9%, respectively. The results demonstrate that all validation parameters were within acceptable ranges for the analytical purposes.

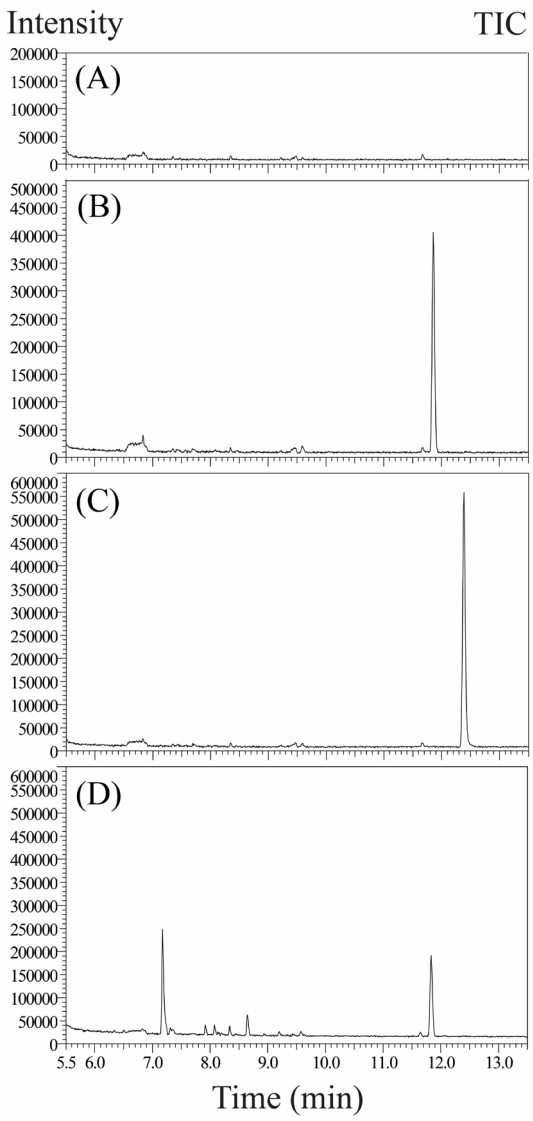

Figure 1. Representative GC-MS chromatograms of (A) blank hexane, (B) terpinen-4-ol solution (1.0 µg/mL), (C) IS (1.2 µg/mL), and (D) essential oil (1.75 µg/mL)
Note: The peaks at 11.87 and 12.40 min were for terpinen-4-ol and IS, respectively.

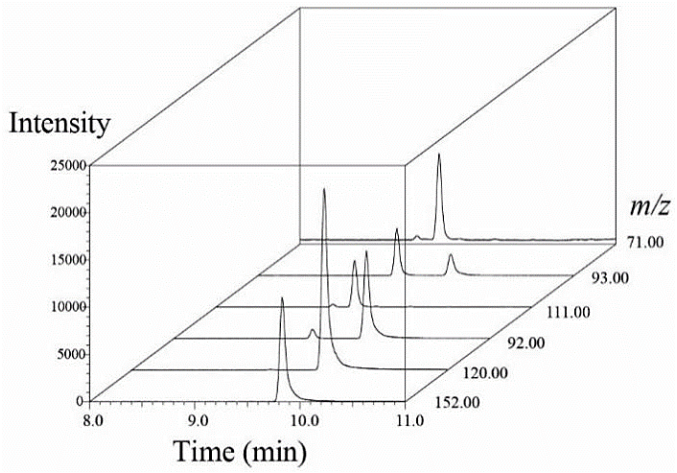

Figure 2. SIM chromatograms of plai oil (140 ng/mL) spiked with IS (360 ng/mL)

3.2 Determination of T4 content in the plai oil

The validated GC-MS method was applied for the determination of T4 content in plai oil. The T4 content was 21.23% w/w. The representative chromatogram of the plai oil is shown in Figure 3(A), indicating that the oil contained at least 13 compounds. All compounds were eluted within 12.0 min. Based on the percentage peak area, the major compounds were sabinene (36.18%), terpinen-4-ol (33.72%), γ-terpinene (9.20%), α-terpinene (4.58%), and α-pinene (1.33%), which fell within the acceptance criteria of the Thai industrial standard (Table 1) (Thai Industrial Standards Institute, 2000).

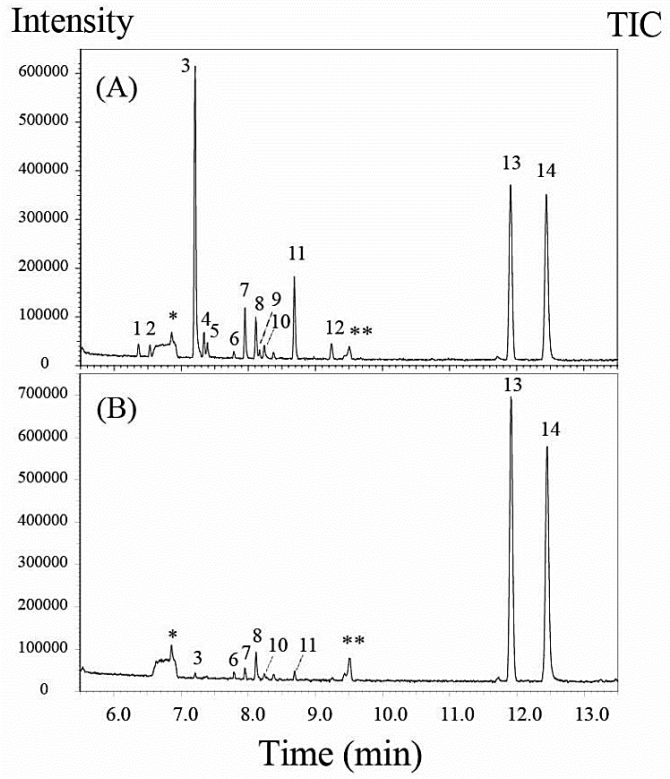

Figure 3. Typical GC-MS chromatogram of (A) plai oil spiked with IS and (B) deionized water from the solubility study spiked with IS
Note: (1) α-phellandrene, (2) α-pinene, (3) sabinene, (4) β-pinene, (5) β-myrcene, (6) α-thujene, (7) α-terpinene, (8) dl-limonene, (9) p-cymene, (10) β-phellandrene, (11) γ-terpinene, (12) α-terpinolene, (13) terpinen-4-ol, (14) IS. Asterisks (*, **) indicate unknown peaks that may originate from the solvent.

Table 1. Percentage relative peak area of the plai oil components used in this study and the acceptance criteria for the major components according to the Thai industrial standard

Component	Compositions based on peak area (%)	
	Plai oil[a]	Thai industrial standard
α-phellandrene	1.11 ± 0.04	-
α-pinene	1.33 ± 0.02	1.00–3.00
α-terpinene	4.58 ± 0.04	3.00–8.00
α-terpinolene	1.69 ± 0.04	-
α-thujene	0.59 ± 0.001	-
β-myrcene	1.98 ± 0.07	-
β-phellandrene	1.50 ± 0.08	-
β-pinene	2.79 ± 0.10	-
dl-limonene	1.59 ± 0.06	-
γ-terpinene	9.20 ± 0.29	6.00–10.00
p-cymene	3.73 ± 0.14	-
sabinene	36.18 ± 0.20	31.00–48.00
terpinen-4-ol	33.72 ± 0.39	19.00–36.00

[a] Data are expressed as mean ± SD

3.3 Solubility of T4

Figure 4 shows the solubility of T4 in its pure form and in plai oil in deionized water and in Ringer's solution. The solubility of T4 in its pure form was 3.51 ± 0.05 µg/mL in deionized water and 5.03 ± 0.06 µg/mL in Ringer's solution, both of which were independent of the initial concentrations. For plai oil, the solubility in deionized water and Ringer's solution ranged from 1.12 ± 0.02 to 1.44 ± 0.01 and 1.14 ± 0.02 to 1.51 ± 0.01 µg/mL, respectively, which gradually increased with higher initial concentrations.

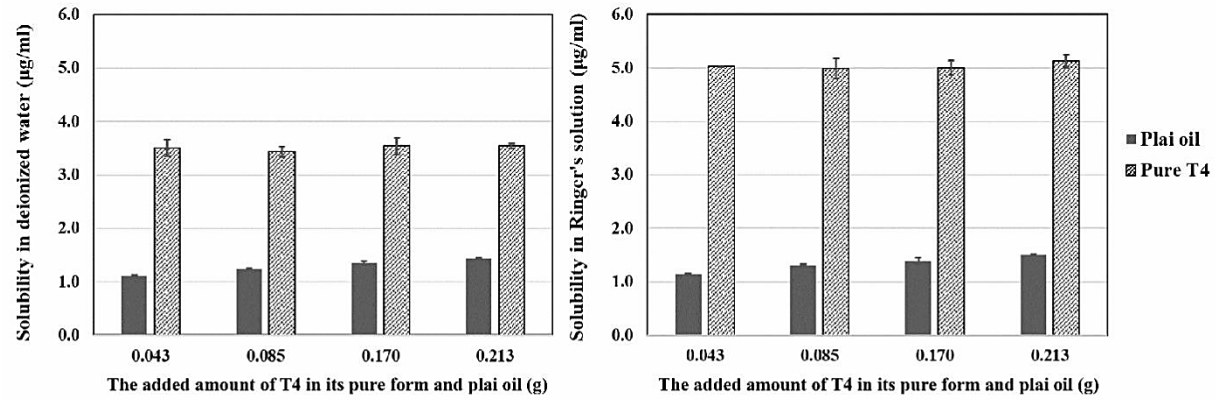

Figure 4. Aqueous solubility of T4 in its pure form and plai oil

3.4 Partition coefficient of T4

The log K_{ow} of pure T4 were 0.73, 1.19, 1.32, and 2.83, while those for plai oil were 0.77, 1.31, 1.56, and 2.88, when the octanol concentration of T4 was 0.125, 0.5, 1.0, and 2.0 mg/mL, respectively. The values obtained from both forms were similar and concentration-dependent, and they increased with increasing initial concentration. From a previous study, the log K_{ow} values of pure T4 determined using reversed-phase HPLC and the shake-flask method were 3.26 and 2.8, respectively (Griffin et al., 1999). Variations in values may result from differences in the testing method and conditions, including the studied concentrations.

3.5 Monte Carlo simulation

The results of the PK parameters from the raw dermal concentration data provided in the supplementary file using the non-compartmental model in the Phoenix64 WinNonlin® software are summarized in Table 2. The steady-state PK parameters of T4 predicted from the one-compartment model in the Oracle Crystal Ball® software using MCS after topical administration of T4 (1.0, 1.9, and 3.8 mg/cm²) are summarized in Table 3. The $AUC_{0\text{-last}}$ and $AUC_{0\text{-infinity}}$ presented in both the non-compartment model from the raw data of the dermal concentration of T4 and the one-compartment model from the Monte Carlo simulation were in a similar range, although the C_{max} was found to be slightly lower in the one-compartment model for all dosages. Since the C_{max} values in those three regimens were quite lower than the MIC values reported in previous studies, further dosing simulations were necessary to cover the MIC values up to 1,489.6 (*C. acnes*) and 2,327.5 µg/mL (*S. aureus*). The concentration-time profile demonstrated linear pharmacokinetics for all dosages (1.0, 1.9, and 3.8 mg/cm²). Therefore, the PK parameters (V_d, CL, k_e) calculated from the dermal concentration of T4 at 1.0 mg/cm² were applied for the MCS using the one-compartment model in the Oracle Crystal Ball® software for further dosing regimen prediction. The %PTA of simulated T4 dosing regimens with different intervals to cover the MIC ranging from 100–1,489.6 µg/mL are shown in Figures 5–7.

Table 2. PK parameters were calculated from dermal T4 concentration using the non-compartmental model in Phoenix64 WinNonlin® software

PK parameters	Dermal T4 concentration		
	1 mg/cm²	**1.9 mg/cm²**	**3.8 mg/cm²**
C_{max} (µg/mL)	4.83	8.04	10.45
T_{max} (h)	0.50	0.80	0.80
$AUC_{0\text{-last}}$ (µg.h/mL)	5.38	11.28	16.25
$AUC_{0\text{-infinity}}$ (µg.h/mL)	5.40	11.32	16.26
k_e (h^{-1})	1.15	1.14	0.98
$t_{1/2}$ (h)	0.60	0.60	0.70
V_d (L)	0.16	0.15	0.20
CL (L/h)	0.19	0.17	0.23

Table 3. PK parameters at steady state predicted from Monte Carlo simulations using the one-compartment model in Oracle Crystal Ball® software

PK parameters	Dermal T4 concentration		
	1 mg/cm²	**1.9 mg/cm²**	**3.8 mg/cm²**
C_{max} (µg/mL)	2.77	5.26	7.89
T_{max} (h)	0.75	0.75	0.75
$AUC_{0\text{-last}}$ (µg.h/mL)	5.80	11.02	16.52
$AUC_{0\text{-infinity}}$ (µg.h/mL)	5.80	11.02	16.53

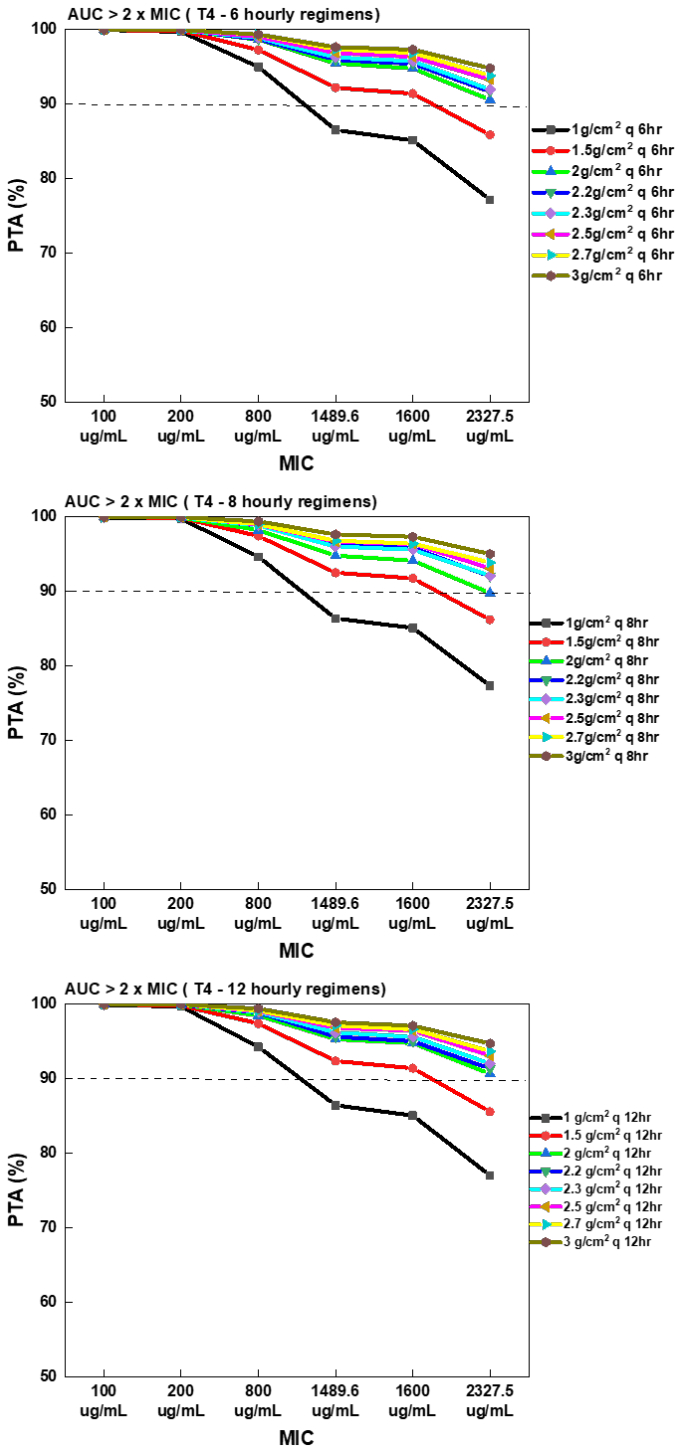

Figure 5. %PTA of T4 against MIC from 100 to 1489.6 µg/mL at the target area under the curve > 2×MIC

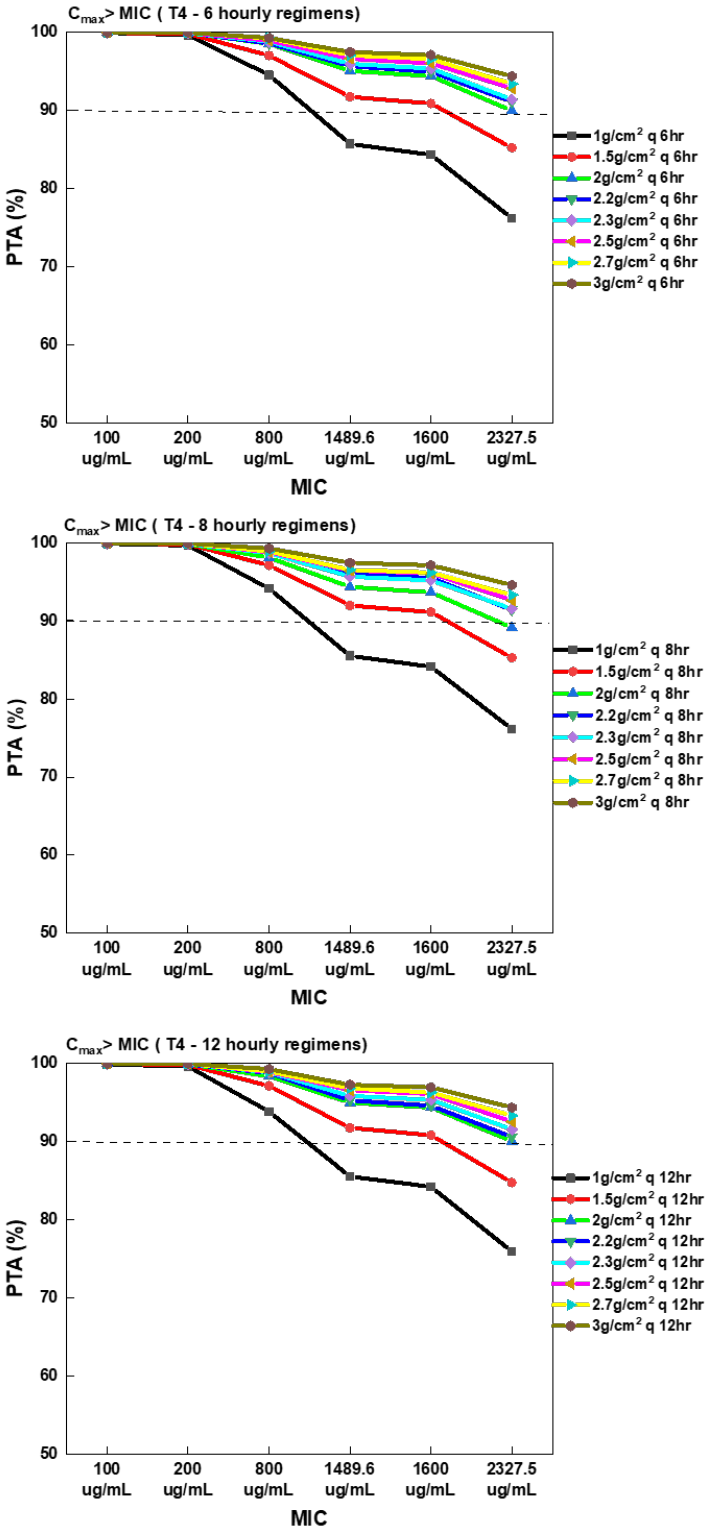

Figure 6. %PTA of T4 against MIC from 100 to 1,489.6 µg/mL at target C_{max} > MIC

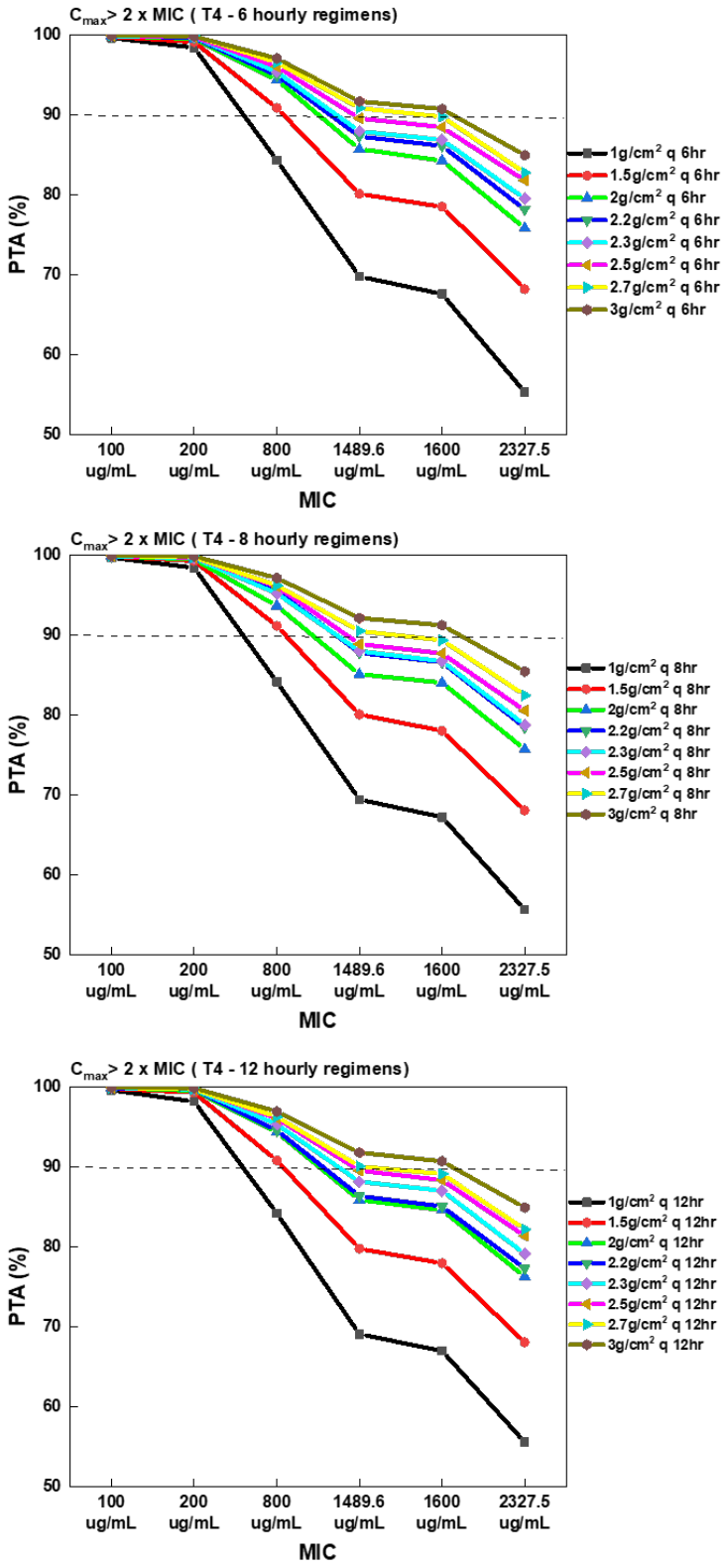

Figure 7. %PTA of T4 against MIC from 100 to 1,489.6 µg/mL at target C_{max} > 2×MIC

3.5.1 %PTA of T4 against MICs of 100, 200, 800, 1,489.6, 1,600, and 2,327.5 µg/mL

Figures 5, 6, and 7 illustrate the %PTA results of T4 in various simulated regimens against MIC ranging from 100 to 2,327.5 µg/mL by focusing on the PKPD targets of AUC > 2×MIC, C_{max} > MIC, and C_{max} > 2×MIC, respectively. Detailed information on the %PTA for all dosing regimens is presented in the supplementary file.

%PTA predicted from the PKPD target (AUC > 2×MIC): All T4 dosage regimens provided a %PTA >90% up to MIC 800 µg/mL by considering the PKPD target of AUC > 2×MIC. However, the %PTA decreased when the MICs were high. A T4 of 1.50 g/cm^2 was required to achieve 90% PTA for MIC 1,600 µg/mL, whereas 2.00 g/cm^2 was needed for MIC 2,327.5 µg/mL. The dosing intervals (6, 8, and 12 h) did not influence the %PTA because all of them showed similar results.

%PTA predicted from the PKPD target of C_{max} > MIC: The calculated outputs of the %PTA using the PKPD target of C_{max} > MIC showed similar patterns as those presented in the results for the AUC > 2×MIC target. All T4 dosage regimens provided %PTA >90% up to MIC 800 µg/mL, but the %PTA decreased with higher MICs. A T4 of 1.50 g/cm^2 was required to achieve 90% PTA for MIC 1,600 µg/mL, whereas 2.00 g/cm^2 was necessary for MIC 2,327.5 µg/mL. The dosing intervals (6, 8, and 12 h) did not affect the %PTA because all of them showed similar results.

%PTA predicted from the PKPD target of C_{max} > MIC and C_{max} > 2×MIC: The simulation outputs of the %PTA using the PKPD target of C_{max} > 2×MIC showed different patterns compared with the results of the AUC > 2×MIC and C_{max} > MIC targets. All T4 dosage regimens provided >90% %PTA up to MIC 200 µg/mL, but the %PTA decreased with higher MICs. A T4 of 1.50 g/cm^2 was required to achieve 90% PTA for MIC 800 µg/mL. In addition, a T4 level of 2.70 g/cm^2 was required to suppress the MIC 1,489.6 µg/mL. The maximum T4 dose of 3.00 g/cm^2 was needed to achieve 90% PTA of the MIC 1,600 µg/mL. Nevertheless, a T4 dose of 3.00 g/cm^2 could suppress the MIC 2,327.5 µg/mL, achieving approximately 85% PTA. The dosing intervals (6, 8, and 12 h) did not affect the %PTA predictions.

3.5.2 Topical dosage regimens for T4 and plai oil

From all dosage regimen simulation results, it can be noted that T4 suppressed the activity of *C. acnes* and *S. aureus* in different manners. To effectively inhibit *C. acnes*, considering the 90% PTA and PKPD targets of AUC > 2×MIC and C_{max} > MIC, a T4 dosage of 1.50 g/cm^2 every 12 h was needed to suppress MIC values up to 1,489.6 µg/mL. Nevertheless, if we considered the 90% PTA and focused on the PKPD target of C_{max} > 2×MIC, a T4 dosage regimen of 2.70 g/cm^2 every 12 h was required to suppress MIC 1,489.6 µg/mL. To inhibit the activity of *S. aureus*, considering the 90% PTA and focusing on the PKPD target of C_{max} > 2×MIC, a T4 dosage regimen of 2.70 g/cm^2 every 12 h was necessary to suppress MIC 1,600 µg/mL. Unfortunately, the targeted MIC of *S. aureus* in this study was 2,327.5 µg/mL. Therefore, a T4 dosage regimen of 3.00 g/cm^2 every 12 h was necessary to cover MIC 2,327.5 µg/mL. Based on the plai oil containing 21.23% w/w T4, the topical dosage regimen conversion from T4 to the plai oil is presented in Table 4.

Table 4. Topical predicted dosage regimens of T4 and plai oil against *C. acnes* and *S. aureus*

PKPD target	MIC 1,489.6 µg/mL (MIC of *C. acnes*)		MIC 2,327.5 µg/mL (MIC of *S. aureus*)	
	T4	Plai oil	T4	Plai oil
AUC > MIC = 2	1.50 g/cm^2 q 12 h (90%PTA)	7.07 g/cm^2 q 12 h (90%PTA)	2.00 g/cm^2 q 12 h (90%PTA)	9.42 g/cm^2 q 12 h (90%PTA)
C_{max} > MIC = 1	1.50 g/cm^2 q 12 h (90%PTA)	7.07 g/cm^2 q 12 h (90%PTA)	2.00 g/cm^2 q 12 h (90%PTA)	9.42 g/cm^2 q 12 h (90%PTA)
C_{max} > MIC = 2	2.70 g/cm^2 q 12 h (90%PTA)	12.72 g/cm^2 q 12 h (90%PTA)	3.00 g/cm^2 q 12 h (85%PTA)	14.13 g/cm^2 q 12 h (85%PTA)

4. DISCUSSION

T4 and essential oils containing T4, such as tea tree oil, have been continuously reported for their antibacterial, anti-inflammatory, and anticancer activities (Cordeiro et al., 2020; Cao et al., 2022; Rahman et al., 2023). In this study, we focused on the topical use of T4 and plai oil for the treatment of acne, cutaneous inflammation, and infections.

Topical products were designed for drug permeation across the skin into subcutaneous tissue or local target sites and then clearance by diffusion, metabolism, and dermal circulation to deeper tissues and the rest of the body (Roberts et al., 2021). The stratum corneum (SC) is generally considered the primary barrier for skin permeation. The key factors affecting skin absorption characteristics are skin lipid-disrupting capability, drug-SC miscibility, and partitioning capability (Tian et al., 2021). Generally, a small molecule (M.W.<500 Da), soluble (generally low melting point), and moderately lipophilic (log K_{ow} in the range of 0–5) compound with few hydrogen bonds will readily cross the SC (Roberts et al., 2021).

According to our studies, the solubility of the pure form was higher than that of the essential oil in all the investigated aqueous media. These results might be because the affinity of T4 for essential oils was higher than that of aqueous media. Similarly, the appearance of some terpenes in tea tree oil could reduce the aqueous solubility of T4, resulting in a decrease in its antimicrobial activity (Cox et al., 2001). Therefore, the formulation of plai oil may require more solubilizer than the development of pure T4. More interestingly, our preliminary study of the solubility of plai oil in deionized water demonstrated that the major compound partitioned into deionized water was T4. A GC-MS chromatogram of the aqueous medium in SCAN mode is shown in Figure 4(B). For the partition coefficient study, the log K_{ow} values of T4 in the pure form and plai oil were in the range of 0.73–2.83 and 0.77–2.88, respectively,

indicating that this compound was moderately lipophilic. Therefore, T4 could be considered as a major active compound of plai oil that could partition into hydrophilic tissue fluid and be responsible for several biological activities.

The data from previous studies regarding the dermal pharmacokinetics of T4 following topical application of plai oil and intravenous injection of pure T4 were used in the simulation study. The unbound T4 concentrations in the dermal tissue were measured using microdialysis. The results demonstrated that after topical application of plai oil, T4 penetrated the dermal tissue with a linear absorption in the range of 2–8 mg/cm^2, corresponding to the amount of T4 of 1.0–3.8 mg/cm^2 (Chooluck et al., 2012, 2013).

There is limited research on the pharmacokinetics of T4 extracted from plai oil. Our calculated PK parameters using the non-compartment model produced C_{max} values of 4.83, 8.04, and 10.45 µg/mL, alongside $AUC_{0\text{-infinity}}$ values of 5.40, 11.32, and 16.26 µg·h/mL, resulting from three distinct topical T4 dosing regimens. Remarkably, these values closely align with the findings of a prior dermal pharmacokinetic study led by Chooluck and colleagues (Chooluck et al., 2012). In their research, they used raw plai oil quantities of 2, 4, and 8 mg/cm^2. The recorded C_{max} values were 4.90, 8.09, and 10.68 µg/mL, accompanied by AUC values of 5.31, 11.23, and 16.23 µg·h/mL, as determined through non-compartmental analysis. Notably, Chooluck and colleagues stated that their utilized plai oil contained 48.1% T4, which corresponds to 1.0, 1.9, and 3.8 mg of T4 being equal to 2.08, 3.96, and 7.92 mg of plai oil, respectively. In addition, it is noteworthy that the C_{max} value derived from our one-compartment model using MCS exhibited slight deviations from both the C_{max} values calculated using non-compartmental analysis with Phoenix64 WinNonlin® software and the results reported by Chooluck This variation in C_{max} could result from variations in the absorption rate constant of T4. Nevertheless, the AUC values remained consistent with those of the previous study, thereby providing accurate validation of the dosing simulation procedures.

The MICs of T4 extracted from tea tree oil have been explored in several studies, demonstrating its effectiveness against *S. aureus* and *C. acnes*, with MICs ranging from 0.16% to 0.31% v/v (Raman et al., 1995). Cordeiro and colleagues also revealed that T4 has antibacterial activity against *S. aureus*, with an MIC of 0.25% v/v (Cordeiro et al., 2020). In contrast, investigations into the MICs of plai oil against different bacteria revealed higher values. Pithayanukul and colleagues reported that 5% plai oil gel presented weak antimicrobial activity because it required higher concentrations to effectively eliminate bacteria. Specifically, the MBC of plai oil against *S. aureus* was 79 mg/mL (Pithayanukul et al., 2007).

Currently, studies have not yet examined the MICs of T4 derived from plai oil. However, the T4 concentrations in tea tree oil and plai oil were similar. Hence, we adopted the MICs of active T4 against *C. acnes* and *S. aureus* from previous research and assumed that T4 from plai oil would exhibit similar effects. However, when we applied these MIC values (1,489.6 µg/mL or 0.16% v/v, and 2,327.5 µg/mL or 0.25% v/v), we discovered that they exceeded the C_{max} and AUC values resulting from typical doses of 1.0, 1.9, and 3.8 mg/cm^2. As a result, we conducted further dosing simulations, gradually increasing the T4 dose until the MIC was reached.

The PK/PD indices (C_{max} > MIC, AUC > MIC and T > MIC) are frequently used as targets in dose selection. The PKPD target (the magnitude of the PKPD index) can be obtained from clinical and/or nonclinical studies. However, during the development of new antimicrobial agents, the target of PKPD was derived from nonclinical studies. Typically, nonclinical PKPD target values range from net bacterial stasis to a 2-$\log_{10}$ colony-forming unit (CFU) reduction from baseline. The specific PKPD index and magnitude of T4 against bacteria have not yet been studied in clinical or nonclinical settings. However, Johansen et al. (2022) conducted a time-kill study using different concentrations of a mixture of T4 and alpha terpineol in a 10:1 ratio with concentrations equivalent to MIC, 2×MIC, and 4×MIC. The findings indicated that the combination equivalent to 4×MIC exhibited bactericidal properties against *S. aureus* and other bacterial strains, including MRSA, reducing the bacterial count by 3-$\log_{10}$ within less than an hour (Johansen et al., 2022). When determining appropriate dosing regimens for infections associated with lower bacterial counts, achieving bacterial stasis may be an adequate goal for the PKPD target. However, for infections with higher bacterial counts, a 1-$\log_{10}$ CFU reduction from the initial count could be a suitable endpoint. Therefore, based on these considerations, the estimated PKPD targets for bacteria with high MIC strains—C_{max} > MIC, C_{max} > 2×MIC, and AUC > 2×MIC—could serve as reasonable indicators for the effectiveness of T4. However, considering topical treatment, active drugs are needed to reach body fluids and penetrate tissues at concentrations sufficient to kill or suppress pathogen growth (Cordeiro et al., 2020).

The simulated dosage requirements concerning the highest efficacy and lowest side effects of T4 to suppress *C. acnes* with MIC 1,489.6 µg/mL and *S. aureus* with MIC 2,327.5µg/mL showed that the topical T4 dosage regimens should be 1.50 g/cm^2 and 2.00 g/cm^2, respectively, to achieve the PKPD targets of C_{max} > MIC and AUC > 2×MIC. For plai oil containing 21.23% T4, the topical dosage regimens of plai oil to suppress *C. acnes* and *S. aureus* should be 7.07 g/cm^2 and 9.42 g/cm^2, respectively, every 12 h. At a higher MIC or the anticipating penetration of T4 to suppress *C. acnes* with MIC 1,489.6 µg/mL using C_{max} > 2×MIC as the PKPD target, T4 and plai oil topical dosage regimens should be 2.70 g/cm^2 and 12.72 g/cm^2 every 12 h, respectively. In addition, to suppress *S. aureus* with MIC 2,327.5 µg/mL using C_{max} > 2×MIC as the PKPD target, the T4 and plai oil topical dosage regimens should be 3.00 g/cm^2 and 14.13 g/cm^2 every 12 h, respectively.

Our study used the active compound T4 as the topical dosage form in the simulation by incorporating the dermal pharmacokinetic parameters of T4, the MIC of bacteria, and the pharmacodynamic target to predict the effective dose for *C. acnes* and *S. aureus* at specific MICs. The predicted outcomes (effective dose for specific bacteria) might be helpful for future research on the formulation design of topical T4 and plai oils.

According to the log K_{ow} values of T4 and plai oil, both are moderately lipophilic and can effectively partition into the lipid-rich outermost layer of the skin, the SC. This property facilitates drug absorption through the skin and its distribution across different skin layers, including the epidermis and dermis. The log K_{ow} values indicate that T4 and plai oil can be formulated in various topical dosage

forms, such as creams, gels, ointments, and lotions. Enhancers, such as emulsifiers, can be used to optimize drug delivery.

Pithayanukul et al. (2007) prepared a 5% plai gel formulation and conducted in vitro tests to determine its antibacterial activity as a naturally occurring antibacterial agent for skin infections. A study by Limwattananon and colleagues used 1% plai oil gel to investigate the efficacy and safety profile of plai gel in treating mild to moderate acne vulgaris (Limwattananon et al., 2008). Additionally, another study successfully developed a clear gel formulation of plai oil, incorporating Carbopol 940 and poly (vinyl alcohol) (Janpim et al., 2011).

The skin, which is the outermost covering of the body, is the primary barrier to drug permeation. To enhance drug permeation and improve therapeutic efficacy, a suitable carrier system, such as a microemulsion, is highly desirable because it facilitates drug transfer through the skin by overcoming these barriers (Shukla et al., 2018). Previous studies have shown that, in addition to gel formulations, plai oil has been successfully prepared in oil-in-water emulsions to enhance the penetration of active compounds through the skin barrier and improve stability (Surassmo et al., 2013).

The content of T4 in plai oil can vary slightly depending on the extraction method used (Sukatta et al., 2009; Singsai et al., 2022). Other studies also reported that the percentage of T4 from plai oil is around 32%–48.1% (Pithayanukul et al., 2007; Chooluck et al., 2012). The percentage of T4 quantified from plai oil purchased from Kovic Kate International Co. Ltd. was within this range, at 33.72%. Additionally, the added base in the formulation may alter the T4 concentration in the final product. Therefore, future research should ensure an accurate content of T4 in the plai oil used to achieve the predicted effective dose in the final product.

As our study is only for simulating the effective dosage of T4 and plai oil for future formulation research, toxicity and skin irritation studies are needed in the future. There are limited studies on the safety of plai oil. A previous study by Songkro et al. (2008) examined how essential oils from *Zingiber officinale*, *Zingiber cassumunar*, and *Curcuma zedoaria* affected diclofenac sodium absorption in Wistar rat skin. The oils were tested at 1%, 3%, and 5% in a hydroalcoholic mixture, which significantly enhanced diclofenac absorption. The effectiveness was ranked as *Zingiber officinale* > *Zingiber cassumunar* > *Curcuma zedoaria*, with 5% oil showing the highest enhancement. *In vivo* irritation tests using 1%, 5%, and 100% oil concentrations revealed varying degrees of skin irritation, with 1% causing severe damage and 5% causing mild irritation (Songkro et al., 2008). However, one study on mild to moderate acne vulgaris using 1% plai oil gel reported that it is safe and no adverse events were observed (Limwattananon et al., 2008).

The limitations of our study were considered to be four items. First, the dermal T4 concentrations investigated via microdialysis in rats may differ from the human T4 concentration. Second, the MIC of T4 in previous studies may deviate from the actual skin infection in a specific population. Third, the PKPD targets were predicted based on simulation; therefore, the time-kill study of pure T4 from plai oil against *C. acnes* is necessary to obtain the actual PKPD targets for dosage regimen prediction. Finally, the toxicity and safety profiles of T4 and plai oil were not assessed in this study because it focused solely on simulating the effective dosage of T4 and plai oil for future formulation research. The log K_{ow} values of T4 and plai oil indicate that their moderate lipophilicity reduces the risk of excessive systemic absorption, potentially limiting systemic side effects and improving the safety profile of topical formulations. Therefore, future research on the formulation of T4 and plai oil should include clinical trials to evaluate the potential for skin irritation or sensitization. Despite the few limitations, our study provides valuable information for further research to determine the safe and effective topical dosage regimens of T4 in clinical study settings.

5. CONCLUSION

Our study developed and validated an accurate, simple, and reproducible GC-MS method to determine T4 in plai oil and investigated its solubility and partition coefficient. The MCS were also conducted to predict the optimal antimicrobial dosage regimens for T4 against *C. acnes* and *S. aureus* with different MICs. Results showed that to suppress *C. acnes* with MIC 1,489.6 µg/mL and *S. aureus* with MIC 2,327.5 µg/mL, the topical T4 dosage regimens should be 1.50 g/cm^2 and 2.00 g/cm^2, respectively, to achieve the PKPD targets of C_{max} > MIC and AUC > 2×MIC. The dosing conversion to topical dosage regimens of plai oil to suppress *C. acnes* and *S. aureus* should be 4.45 g/cm^2 and 5.93 g/cm^2, respectively, every 12 h. When considering the higher MIC or the anticipated penetration of T4 to suppress *C. acnes* with MIC 1,489.6 µg/mL using C_{max} > 2×MIC as the PKPD target, the T4 and plai oil topical dosage regimens should be 2.70 g/cm^2 and 8 g/cm^2 every 12 h, respectively. In addition, to suppress *S. aureus* with MIC 2,327.5 µg/mL using C_{max} > 2×MIC as the PKPD target, the T4 and plai oil topical dosage regimens should be 3 g/cm^2 and 8.9 g/cm^2 every 12 h, respectively. The overall physicochemical and simulation results will help in future topical formulation design for pure T4 and plai oils.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Pharmacometrics Center, Faculty of Pharmacy, Mahidol University, for providing research facilities, and the financial support from the Reinventing University project of the Ministry of Higher Education, Science, Research and Innovation, Thailand, for this research work.

REFERENCES

Alshiekheid, M. A., Dwiningsih, Y., Sabour, A. A., & Alkahtani, J. (2022). Phytochemical composition and antibacterial activity of *Zingiber cassumunar* Roxb. against agricultural and foodborne pathogens. *Preprints*, Article 2022080511. https://doi.org/10.20944/Preprints202208.0511.v1

AOAC. (2002). *Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals*. Association of Official Analytical Chemistry. https://s27415.pcdn.co/wp-content/uploads/2020/01/64ER20-7/Validation_Methods/d-AOAC_Guidelines_For_Single_Laboratory_Validation_Dietary_Supplements_and_Botanicals.pdf

Asín-Prieto, E., Rodríguez-Gascón, A., & Isla, A. (2015). Applications of the pharmacokinetic/pharmacodynamic (PK/PD) analysis of antimicrobial agents. *Journal of Infection and Chemotherapy*, *21*(5), 319–329. https://doi.org/10.1016/j.jiac.2015.02.001

Cal, K. (2006). Aqueous solubility of liquid monoterpenes at 293 K and its relationship with the calculated log P value. *Yakugaku Zasshi*, *126*(4), 307–309. https://doi.org/10.1248/yakushi.126.307

Cao, W., Tian, R., Pan, R., Sun, B., Xiao, C., Chen, Y., Zeng, Z., & Lei, S. (2022). Terpinen-4-ol inhibits the proliferation and mobility of pancreatic cancer cells by downregulating Rho-associated coiled-coil containing protein kinase 2. *Bioengineered*, *13*(4), 8643–8656. https://doi.org/10.1080/21655979.2022.2054205

Carson, C. F., Hammer, K. A., & Riley, T. V. (2006). *Melaleuca alternifolia* (Tea Tree) oil: A review of its antimicrobial and other medicinal properties. *Clinical Microbiology Reviews*, *19*(1), 50–62. https://doi.org/10.1128/CMR.19.1.50-62.2006

Chongmelaxme, B., Sruamsiri, R., Dilokthornsakul, P., Dhippayom, T., Kongkaew, C., Saokaew, S., Chuthaputti, A., & Chaiyakunapruk, N. (2017). Clinical effects of *Zingiber cassumunar* (Plai): A systematic review. *Complementary Therapies in Medicine*, *35*, 70–77. https://doi.org/10.1016/j.ctim.2017.09.009

Chooluck, K., Singh, R. P., Sathirakul, K., & Derendorf, H. (2012). Dermal pharmacokinetics of terpinen-4-ol following topical administration of *Zingiber cassumunar* (Plai) oil. *Planta Medica*, *78*(16), 1761–1766. https://doi.org/10.1055/s-0032-1315262

Chooluck, K., Singh, R. P., Sathirakul, K., & Derendorf, H. (2013). Plasma and dermal pharmacokinetics of terpinen-4-ol in rats following intravenous administration. *Die Pharmazie*, *68*(2), 135–140. https://doi.org/10.1691/ph.2013.2116

Cordeiro, L., Figueiredo, P., Souza, H., Sousa, A., Andrade-Júnior, F., Medeiros, D., Nóbrega, J., Silva, D., Martins, E., Barbosa-Filho, J., & Lima, E. (2020). Terpinen-4-ol as an antibacterial and antibiofilm agent against *Staphylococcus aureus*. *International Journal of Molecular Sciences*, *21*(12), Article 4531. https://doi.org/10.3390/ijms21124531

Cox, S. D., Mann, C. M., & Markham, J. L. (2001). Interactions between components of the essential oil of *Melaleuca alternifolia*. *Journal of Applied Microbiology*, *91*(3), 492–497. https://doi.org/10.1046/j.1365-2672.2001.01406.x

Dong, L., Liu, C., Cun, D., & Fang, L. (2015). The effect of rheological behavior and microstructure of the emulgel on the release and permeation profiles of Terpinen-4-ol. *European Journal of Pharmaceutical Sciences*, *78*, 140–150. https://doi.org/10.1016/j.ejps.2015.07.003

Griffin, S., Wyllie, S. G., & Markham, J. (1999). Determination of octanol-water partition coefficient for terpenoids using reversed-phase high-performance liquid chromatography. *Journal of Chromatography A*, *864*(2), 221–228. https://doi.org/10.1016/S0021-9673(99)01009-2

Han, A. R., Kim, H., Piao, D., Jung, C. H., & Seo, E. K. (2021). Phytochemicals and bioactivities of *Zingiber cassumunar* Roxb. *Molecules (Basel, Switzerland)*, *26*(8), Article 2377. https://doi.org/10.3390/molecules26082377

Hart, P., Brand, C., Carson, C., Riley, T. V., Prager, R. H., & Finlay-Jones, J. J. (2000). Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. *Inflammation Research*, *49*(11), 619–626. https://doi.org/10.1007/s000110050639

Hazarika, N. (2021). Acne vulgaris: New evidence in pathogenesis and future modalities of treatment. *Journal of Dermatological Treatment*, *32*(3), 277–285. https://doi.org/10.1080/09546634.2019.1654075

Janpim, K., Sakkumduang, W., Nualkaew, S., & Priprem, A. (2011, October 24–25). *Development of plai* [Conference session]. The 2nd International Conference on Applied Science (ICAS) and the 3rd International Conference on Science and Technology for Sustainable Development of The Greater Mekong Sub-Region (STGMS). Souphanouvong University, Luang Prabang, Lao.

Johansen, B., Duval, R. E., & Sergere, J.-C. (2022). First evidence of a combination of terpinen-4-ol and α-terpineol as a promising drug against ESKAPE pathogens. *Molecules*, *27*(21), Article 7472. https://doi.org/10.3390/molecules27217472

Karadag, A. S., Aslan Kayıran, M., Wu, C.-Y., Chen, W., & Parish, L. C. (2021). Antibiotic resistance in acne: Changes, consequences and concerns. *Journal of the European Academy of Dermatology and Venereology*, *35*(1), 73–78. https://doi.org/10.1111/jdv.16686

Kirschbaum, J. O., & Kligman, A. M. (1963). The pathogenic role of *Corynebacterium Acnes* in acne vulgaris. *JAMA Dermatology*, *88*(6), 832–833. https://doi.org/10.1001/archderm.1963.01590240156026

Lee, C.-J., Chen, L.-W., Chen, L.-G., Chang, T.-L., Huang, C.-W., Huang, M.-C., & Wang, C.-C. (2013). Correlations of the components of tea tree oil with its antibacterial effects and skin irritation. *Journal of Food and Drug Analysis*, *21*(2), 169–176. https://doi.org/10.1016/j.jfda.2013.05.007

Legiawati, L., Halim, P. A., Fitriani, M., Hikmahrachim, H. G., & Lim, H. W. (2023). Microbiomes of acne vulgaris and their susceptibility to antibiotics in Indonesia: A systematic review and meta-analysis. *Antibiotics*, *12*(1), Article 145. https://doi.org/10.3390/antibiotics12010145

Limwattananon, C., Rattanachotphanit, T., Cheawchanwattana, A., Waleekhachonloet, O., Giwanon, R., Choonhakarn, C., Sripanidkulchai, B., & Sakolchai, S. (2008). Clinical efficacy of Plai gel containing 1% plai oil for treating mild to moderate acne vulgaris. *Isan Journal of Pharmaceutical Sciences*, *4*(2), 121–133. https://doi.org/10.14456/ijps.2008.23

Mondello, F., Fontana, S., Scaturro, M., Girolamo, A., Colone, M., Stringaro, A., Vito, M. D., & Ricci, M. L. (2022). Terpinen-4-ol, the main bioactive component of tea tree oil, as an innovative antimicrobial agent against *Legionella pneumophila*. *Pathogens*, *11*(6), Article 682. https://doi.org/10.3390/pathogens11060682

Nurzyńska-Wierdak, R., Pietrasik, D., & Walasek-Janusz, M. (2023). Essential oils in the treatment of various types of acne—A review. *Plants*, *12*(1), Article 90. https://doi.org/10.3390/plants12010090

Pithayanukul, P., Tubprasert, J., & Wuthi-Udomlert, M. (2007). *In vitro* antimicrobial activity of *Zingiber cassumunar* (Plai) oil and a 5% plai oil gel. *Phytotherapy Research*, *21*(2), 164–169. https://doi.org/10.1002/ptr.2048

Rahman, M. A., Sultana, A., Khan, M. F., Boonhok, R., & Afroz, S. (2023). Tea tree oil, a vibrant source of neuroprotection via neuroinflammation inhibition: A critical insight into repurposing *Melaleuca alternifolia* by unfolding its

characteristics. *Journal of Zhejiang University-Science B*, *24*(7), 554–573. https://doi.org/10.1631/jzus.B2300168

Raman, A., Weir, U., & Bloomfield, S. F. (1995). Antimicrobial effects of tea-tree oil and its major components on *Staphylococcus aureus*, *Staph. epidermidis* and *Propionibacterium acnes. Letters in Applied Microbiology*, *21*(4), 242–245. https://doi.org/10.1111/j.1472-765X.1995.tb01051.x

Roberts, J. A., Kirkpatrick, C. M., & Lipman, J. (2011). Monte Carlo simulations: Maximizing antibiotic pharmacokinetic data to optimize clinical practice for critically ill patients. *Journal of Antimicrobial Chemotherapy*, *66*(2), 227–231. https://doi.org/10.1093/jac/dkq449

Roberts, M. S., Cheruvu, H. S., Mangion, S. E., Alinaghi, A., Benson, H. A. E., Mohammed, Y., Holmes, A., van der Hoek, J., Pastore, M., & Grice, J. E. (2021). Topical drug delivery: History, percutaneous absorption, and product development. *Advanced Drug Delivery Reviews*, *177*, Article 113929. https://doi.org/10.1016/j.addr.2021.113929

Sharifi-Rad, J., Salehi, B., Varoni, E. M., Sharopov, F., Yousaf, Z., Ayatollahi, S. A., Kobarfard, F., Sharifi-Rad, M., Afdjei, M. H., Sharifi-Rad, M., & Iriti, M. (2017). Plants of the *Melaleuca* genus used as antimicrobial agents: From farm to pharmacy. *Phytotherapy Research*, *31*(10), 1475–1494. https://doi.org/10.1002/ptr.5880

Shukla, T., Upmanyu, N., Agrawal, M., Saraf, S., Saraf, S., & Alexander, A. (2018). Biomedical applications of microemulsion through dermal and transdermal route. *Biomedicine & Pharmacology*, *108*, 1477–1494. https://doi.org/10.1016/j.biopha.2018.10.021

Singsai, K., Charoongchit, P., & Utsintong, M. (2022). The comparison of the oil types in Plai (*Zingiber cassumunar*) oil extraction and analysis of the chemical constituents in plai oil by gas chromatography-mass spectrometry technique. *Health Science, Science and Technology Reviews*, *15*(3), 18–28. [in Thai]

Songkro, S., Wungsintaweekul, J., & Chartwaingam, S. (2008). Investigation of the enhancing activity and skin irritation of *Zingiber officinale*, *Zingiber cassumunar*, and *Curcuma zedoaria*. *Journal of Drug Delivery Science and Technology*, *18*(3), 169–179. https://doi.org/10.1016/S1773-2247(08)50033-5

Sukatta, U., Rugthaworn, P., Punjee, P., Chidchenchey, S., & Keeratinijakal, V. (2009). Chemical composition and physical properties of oil from Plai (*Zingiber cassumunar* Roxb.) obtained by hydro distillation and hexane extraction. *Agriculture and Natural Resources*, *43*(5), 212–217.

Surassmo, S., Srinuanchai, W., Yostawonkul, J., & Ruktanonchai, U. (2013). Formulation development of plai nanoemulsion based on the influence of surfactant combinations. *Chiang Mai Journal of Science*, *40*(6), 994–999.

Tandirogang, N., Anitasari, S., Arung, E. T., Paramita, S., & Shen, Y. K. (2022). Evaluation of the antibacterial properties of *Zingiber purpureum* essential oil against 13 different gram-positive and gram-negative bacteria. *Indonesian Biomedical Journal*, *14*(3), 303–308. https://doi.org/10.18585/inabj.v14i3.1967

Thai Industrial Standards Institute. (2019). *Thai industrial standard for Phlai essential oil (TIS 1679–2541)*. Ministry of Industry. https://service.tisi.go.th/tisi-standard-shop/item/tis/4575 [in Thai]

Tian, Q., Quan, P., Fang, L., Xu, H., & Liu, C. (2021). A molecular mechanism investigation of the transdermal/topical absorption classification system based on drug skin permeation and skin retention. *International Journal of Pharmaceutics*, *608*, Article 121082. https://doi.org/10.1016/j.ijpharm.2021.121082

Vilas-Boas, S. M., da Costa, M. C., Coutinho, J. A. P., Ferreira, O., & Pinho, S. P. (2022). Octanol–water partition coefficients and aqueous solubility data of monoterpenoids: Experimental, modeling, and environmental distribution. *Industrial & Engineering Chemistry Research*, *61*(8), 3154–3167. https://doi.org/10.1021/acs.iecr.1c04196

Vinks, A. A., Derendorf, H., & Mouton, J. W. (2014). *Fundamentals of antimicrobial pharmacokinetics and pharmacodynamics*. Springer. https://doi.org/10.1007/978-0-387-75613-4

Walsh, T. R., Efthimiou, J., & Dréno, B. (2016). Systematic review of antibiotic resistance in acne: An increasing topical and oral threat. *The Lancet Infectious Diseases*, *16*(3), e23–e33. https://doi.org/10.1016/S1473-3099(15)00527-7

Yasin, M., Younis, A., Javed, T., Akram, A., Ahsan, M., Shabbir, R., Ali, M. M., Tahir, A., El-Ballat, E. M., Sheteiwy, M. S., Sammour, R. H., Hano, C., Alhumaydhi, F. A., & El-Esawi, M. A. (2021). River tea tree oil: Composition, antimicrobial and antioxidant activities, and potential applications in agriculture. *Plants*, *10*(10), Article 2105. https://doi.org/10.3390/plants10102105