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Optimization of Tween 80 and PEG-400 Concentration in Indonesian Virgin Coconut Oil Nanoemulsion as Antibacterial against *Staphylococcus aureus*

(Pengoptimuman Kepekatan Tween 80 dan PEG-400 dalam Nanoemulsi Minyak Kelapa Dara Indonesia sebagai Antibakteria terhadap *Staphylococcus aureus*)

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ABSTRACT

Virgin Coconut Oil (VCO) can act as an antibacterial due to free fatty acids. To increase the stability of VCO, the VCO can be formed into nanoemulsion preparations. This study aimed to optimize the concentration of Tween 80 and Polyethylene glycol-400 (PEG-400) in nanoemulsion formula and determine antibacterial activity against *Staphylococcus aureus*. The content of VCO was determined using Gas Chromatography-Mass Spectrometry (GC-MS), and the results showed that VCO contained lauric acid, palmitic acid, caprylic acid, oleic acid, capric acid, and stearic acid. Optimization was carried out using the 2^2 factorial design method on the response of pH, density, percent transmittance, particle size, and polydispersity index. The optimum formula was obtained at concentrations of Tween 80 and PEG-400 40% and 26%, respectively, with a desirability value of 0.961. The optimum formula showed no phase separation and a significant decrease in pH ($p>0.05$). The optimum and comparison formula (pure VCO) had significantly different antibacterial activity ($p<0.05$) where the diameter of the inhibition zone was 24.77±1.66 mm and 16.73±2.00 mm, Minimum Inhibitory Concentration (MIC) of 1250 ppm and 2500 ppm, Minimum Bactericidal Concentration (MBC) of 2500 ppm and more than 2500 ppm, respectively. The optimum formula of VCO nanoemulsion was proven to have good stability and a potent antibacterial activity.

Keywords: Antibacterial; factorial design; nanoemulsion; *Staphylococcus aureus*; virgin coconut oil

ABSTRAK

Minyak Kelapa Dara (VCO) boleh bertindak sebagai antibakteria kerana kandungan asid lemak bebas. Bagi meningkatkan kestabilan VCO, ia boleh disediakan dalam bentuk nano-emulsi. Tujuan kajian ini dijalankan adalah untuk mendapatkan kepekatan yang optimum bagi Tween 80 dan Polietilena glikol-400 (PEG-400) di dalam formulasi nano-emulsi dan untuk menentukan aktiviti antibakteria terhadap *Staphylococcus aureus*. Kandungan VCO telah dianalisis dengan menggunakan Kromatografi gas–spektrometri jisim (GC-MS) dan hasilnya menunjukkan VCO mengandungi asid laurik, asid palmitik, asid kaprilat, asid oleik, asid kaprik dan asid stearik. Pengoptimuman telah dijalankan menggunakan kaedah reka bentuk faktorial 2^2 pada tindak balas pH, ketumpatan, peratus transmisi, saiz zarah dan indeks polidispersiti. Formula optimum didapati pada kepekatan Tween 80 dan PEG-400 masing-masing 40% dan 26%, dengan nilai kemahuan 0.961. Formula optimum juga menunjukkan tiada pemisahan campuran mahupun penurunan pH yang ketara ($p>0.05$). Formula optimum dan perbandingannya (VCO tulen) didapati mempunyai aktiviti antibakteria yang berbeza secara signifikan ($p<0.05$) dengan diameter zon inhibasi masing-masing ialah 24.77±1.66 mm dan 16.73±2.00 mm, Kepekatan Perencatan Minimum (MIC) 1250 ppm dan 2500 ppm dan Kepekatan Bakteria Minimum (MBC) 2500 ppm dan lebih daripada 2500 ppm. Formula optimum nano-emulsi VCO terbukti mempunyai kestabilan yang baik dan aktiviti antibakteria yang tinggi.

Kata kunci: Antibakteria; minyak kelapa dara; nano-emulsi; reka bentuk faktor; *Staphylococcus aureus*

Introduction

Staphylococcus aureus is a Gram-positive pathogenic bacterium. *S. aureus* can cause various health problems such as skin, respiratory, endocarditis, and digestive tract infections (Clark & Hicks 2022; Parker & Prince 2012; Tong et al. 2015). Antibiotics are a widely used treatment in cases of infection. However, many studies have proven that *S. aureus* is resistant to various antibiotics such as penicillin, oxacillin, ampicillin, clindamycin, lincomycin, gentamicin, erythromycin, rifampicin, vancomycin, and trimethoprim (Feßler et al. 2008; Kayili & Sanlibaba 2020; Kumar et al. 2020). The resistance problem encourages the development of antibacterial research derived from natural ingredients.

Virgin Coconut Oil (VCO) is a natural ingredient with antibacterial activity. In high concentrations, VCO contains Medium Chain Fatty acids (MCFA), such as lauric and caprylic acid. MCFA has been shown to inhibit the growth of pathogenic bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, and *Salmonella thyposa* (Abllah & Shahdan 2018; Nakatsuji et al. 2009; Suryani et al. 2020). MCFA can act as an antibacterial by damaging the bacterial cell membrane, causing bacterial lysis (Matsue et al. 2019). The fatty acid can also increase the permeability and immunological function of the skin (Fischer et al. 2020; Man, Elias & Man 2015).

VCO is an oil that is readily oxidized by light, oxygen, heat, and the presence of metals (Sivakanthan, Bopitiya & Madhujith 2018). The oxidation process of oil will produce peroxide compounds which can be further decomposed into aldehydes, ketones, alcohols, and carboxylic acids (Lu & Tan 2009; Rukmini & Raharjo 2010). The oxidation process can impact oil's content, quality, and safety. Nanoemulsion can improve the stability of VCO. Nanoemulsion is an emulsion system with a particle size in the nano-size category, 10-200 nm (Azmi et al. 2019; Gupta et al. 2016). The presence of surfactants and cosurfactants can increase the stability of the nanoemulsion. Surfactants can reduce surface tension, while co-surfactants can prevent aggregation and increase pH stability (Chuacharoen, Prasongsuk & Sabliov 2019). The stability of nanoemulsions can be described by particle size, polydispersity index, zeta potential, and percent transmittance. In this study, Tween 80 was used as a surfactant and PEG-400 as a cosurfactant.

Tween 80 is a non-ionic surfactant with a Hydrophilic-Lipophilic Balance (HLB) value of 15, making it suitable for Oil in Water (O/W) type emulsion preparations. Tween 80 is used in medicinal, cosmetic, and even food preparations as emulsifiers (Nielsen et al. 2016). The higher concentration of Tween 80 will cause the resulting nanoemulsion to have a high percentage of transmittance, clearness, and smaller particle size (Chuacharoen, Prasongsuk & Sabliov 2019; Ujilestari et al. 2018). PEG-400 was used as a cosurfactant to increase the stability of the nanoemulsion. PEG-400 has increased drug absorption and solubility in water (Chen et al. 2019).

Based on these descriptions, the concentration of Tween 80 and PEG-400 was optimized in virgin coconut oil nanoemulsion formula using 2^2 factorial-design where there are two factors (Tween 80 and PEG 400) and two levels, namely the Tween 80 was 40% and 50% and PEG 400 was 13% and 26%. The antibacterial activity against *Staphylococcus aureus* was conducted by measuring the diameter of the inhibition zone and determining the value of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). This test is compared with VCO, which is not made in nanoemulsion preparations.

Materials and Methods

Materials

The materials used were Virgin Coconut Oil from Banyuasin, South Sumatra, Indonesia, VCO (Javara®), *Staphylococcus aureus* ATCC 25923, Tween 80 (Merck®), PEG-400 (Merck®), Nutrient Agar (Merck®), Nutrient Broth (Merck®), and aquadest (Bratachem®), erythromycin (Sigma Aldrich®).

Production of Virgin Coconut Oil

VCO is made by the fishing method. The fishing method is done by adding VCO to coconut cream in a ratio of 1:20. The mixture is stirred slowly and then let stand at 100-120 °C for 10-12 h in a closed and dry container. VCO is separated from coconut cream by filtering method using clean and sterile filter paper (Nurhidayah et al. 2022).

Analysis of VCO Content Using GC-MS

Analysis of VCO content was carried out using GC-MS QP2010 SE with a 30 m × 0.25 mm HP-5 capillary column which was coupled with a mass spectrometer and used Helium as the carrier gas at a column temperature of 80-300 °C (5 °C/min).

PREPARATIONS OF VCO NANOEMULSION

The VCO nanoemulsion formula was designed using 2^2 factorial designs. The factors used are Tween 80 and PEG-400. The higher and lower levels of each factor were represented as (+1) and (-1) and can be seen in Table 1. VCO oil nanoemulsions were prepared using a high-shear stirring method using ultra-turrax (Shehata, Almostafa & Elsewedy 2022). The oil phase (VCO) was added to the water phase (Tween 80, PEG-400, and aquademineral) drop by drop until a clear and transparent nanoemulsion was formed.

EVALUATION OF VCO NANOEMULSION

Organoleptic Test

The test is carried out by observing the colour, odour, and consistency. This observation is done visually.

pH and Density

The pH of the nanoemulsion was measured using a pH meter that had been calibrated using a buffer solution of pH 4.00 and 7.00. The test was carried out in three replications. The density of the nanoemulsion was measured using a pycnometer at room temperature for three replications (Homayoonfal, Khodaiyan & Mousavi 2014).

The Percent Transmittance

The percent transmittance of nanoemulsion was measured using a UV-Vis spectrophotometer at 650 nm. Aquademineral was used as a blank. The test was carried out in three replications (Thakkar et al. 2011).

Particle Size and Polydispersity Index

The particle size and polydispersity index of nanoemulsions were measured using a particle size analyzer (Malvern) at 25 °C with a scattering angle of 90°. The test was carried out in three replications (Apriani, Mardiyanto & Hendrawan 2022).

OPTIMIZATION OF VCO NANOEMULSION

Optimization is carried out using a factorial design approach using the Design Expert 13® tool. The responses used in the optimization process are pH (y1), density (y2), percent transmittance (y3), particle size (y4), and polydispersity index (y5). The first step in the optimization process is fitting the model to the response by looking at the value of R2, adjusted R2, predicted R2, and adequate precision. After that, the optimum formula is determined by looking at the desirability value closest to 1.

STABILITY TEST FOR OPTIMUM FORMULA

Centrifugation and cycling test methods tested the optimum nanoemulsion formula for stability. The centrifugation method was carried out at a speed of 3800 rpm for 5 h. The cycling test method was carried out at 4 °C and 40 °C for 24 h each for six cycles (Mardiyanto, Apriani & Alfarizi 2022).

ANTIBACTERIAL ACTIVITY TEST

The antibacterial activity test was carried out using the diffusion method to determine the diameter of the inhibition zone and the dilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The bacteria used was *Staphylococcus aureus*. In this test, a comparison is used, namely VCO oil at the same concentration as the nanoemulsion. Erythromycin was used as a positive control. The concentration used to determine MIC and MBC is 2500 ppm; 1250 ppm; 625 ppm; 312.5 ppm; 156.25 ppm; and 78.125 ppm (Su'i et al. 2015).

DATA ANALYSIS

Data analysis was performed using Design Expert 13® to see the effect of Tween 80, PEG-400 and their interaction on the evaluation response of nanoemulsions and to determine the optimum formula of VCO nanoemulsion. Meanwhile, the results of antibacterial activity were analyzed using SPSS 25® using the Post Hoc-Duncan method.

RESULTS AND DISCUSSION

PRODUCTION OF VIRGIN COCONUT OIL

VCO is made using the fishing method to separate between VCO, coconut blonde, and the water. The VCO produced in this study is transparent in colour, which can be seen in Figure 1. The content of VCO was analyzed using GC-MS. Based on the results of the analysis, VCO was proven to contain lauric acid (26.25%, peak 3), palmitic acid (7.21%, peak 4), caprylic acid (6.12%, peak 1), oleic acid (5.78%, peak 6), capric acid (5.44%, peak 2), and stearic acid (4.76%, peak 5) (Figure 2). The main content of VCO in this study is lauric acid. Various studies also prove that the lauric acid content in VCO can reach 50% (Boateng et al. 2016; Kenechukwu et al. 2022). VCO content limits in Indonesia are regulated in SNI 7381:2008, where the concentration range of lauric acid is 45.1-53.2%, palmitic acid is 7.5-10.2%, caprylic acid is 4.6-10%, oleic acid is 5-10%, capric acid is 5-8%, and stearic acid (2-4%) (Standar Nasional Indonesia 2008). The difference in the results obtained is influenced by many factors, such as the process of making VCO (Agarwal 2017).

VCO NANOEMULSION

The VCO nanoemulsion consisted of 4 formulas with different concentrations of Tween 80 and PEG-400, as shown in Table 1. The nanoemulsion obtained was transparent yellow except in Formula 1, where turbidity occurred (Figure 3). The results of the evaluation of nanoemulsion preparations can be seen in Table 2. Formulas 2, 3, and 4 meet the requirements for evaluating nanoemulsion preparations: pH in the skin range 4.5-6.5, percent transmittance close to 100%, particle size less than 200 nm, and PDI less than 0.5 (Laxmi et al. 2015; Liu et al. 2019). Formula 1 has a transmittance percentage close to 0%, which is also evidenced by the cloudy organoleptic results of Formula 1.

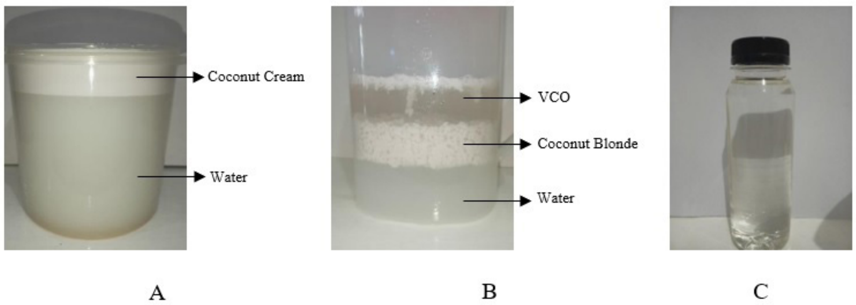

FIGURE 1. The process of making VCO: Formed two layers (A), formed three layers (B), and the results of VCO (C)

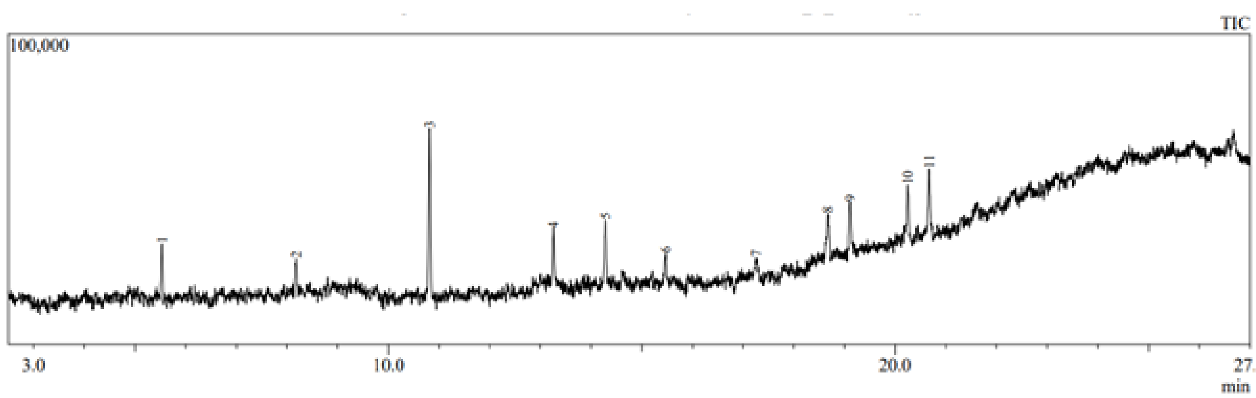

FIGURE 2. GC-MS chromatogram of VCO

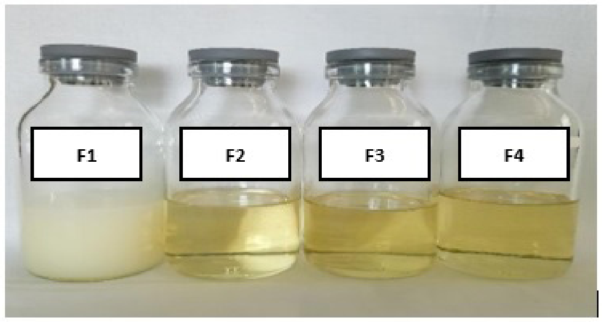

FIGURE 3. Physical appearance of VCO nanoemulsion formulated using four different formulas

TABLE 1. Formula of virgin coconut oil nanoemulsion

Formula	Coded level		Actual level (%)		VCO (%)	Aquademineral (%)
	Tween 80	PEG-400	Tween 80	PEG-400		
1	-1	-1	40	13	10	Ad 50
2	-1	+1	40	26	10	Ad 50
3	+1	-1	50	13	10	Ad 50
4	+1	+1	50	26	10	Ad 50

TABLE 2. The evaluation result of virgin coconut oil nanoemulsion

Parameter	Formula			
	1	2	3	4
Organoleptic	Yellowish White, Turbid	Yellow, Clear	Yellow, Clear	Yellow, Clear
Density (g/mL)	1.050±0.001	1.063±0.001	1.069±0.002	1.076±0.001
pH	6.32±0.02	6.23±0.01	6.31±0.01	6.75±0.01
Transmittance (%)	0.6±0.0	97.3±0.1	98.2±0.1	98.5±0.1
Particle size (nm)	194.30±1.62	14.79±0.09	13.97±0.16	13.79±0.23
PDI	0.082±0.071	0.092±0.008	0.198±0.015	0.102±0.011

MODEL FITTING FOR OPTIMIZATION

Model fitting is done to see how much influence the factors used (Tween 80, PEG-400, and their interaction) on the measured response. Model fitting is done by observing the p-value, F-value, R2, adjusted R2, predicted R2, and adequate precision. The results can be seen in Table 3. A good model for the optimization process must show the influence of factors on the response where the p-value <0.05, F-value is large, R2 is more than 0.7, the difference between adjusted R2 and predicted R2 is less than 0.2, and adequate precision is more than 4 (Apriani, Miksusanti & Fransiska 2022). Based on the results in Table 4, all responses indicate a significant influence. The R2 value obtained from 5 responses ranges from 0.9390-1.000. It means the variation in the results is influenced by 93.90% to 100% of the factors used (Figueiredo, Junior & Rocha 2011). The results of R2 can also be seen from the normal plot of the residuals graph, where the closer to the line, the data is normally distributed, while points far from the line represent the remaining values of R2 (Figure 4). The difference between adjusted R2 and predicted R2 from all responses is less than 0.2, which means that the adjusted R2 value is still within reasonable limits with the predicted R2. Adequate precision describes the ratio between signal and noise. The adequate precision of all responses shows more than four results, indicating the model has an adequate signal (Aziz & Aziz 2018). The F-value suggests the ratio of the effect caused by the factor and the error. The greater the F value, the more significant the influencing factor on the response (Noordin et al. 2004). So, the concentrations of Tween 80 and PEG-400 significantly affect the response of pH, density, percent transmittance, particle size, and polydispersity index.

TABLE 3. The result of fitting model

Response	Regression				ANOVA		
	R^2	Adjusted R^2	Predicted R^2	Adequate precision	p-value	F value	Significance
y_1	0.9981	0.9973	0.9957	83.3165	<0.0001	1377.81	Significant
y_2	0.9927	0.9900	0.9836	45.0333	<0.0001	364.08	Significant
y_3	1.0000	1.0000	1.0000	2626.0382	<0.0001	1.7×10^6	Significant
y_4	0.9999	0.9999	0.9998	380.4920	<0.0001	36038.17	Significant
y_5	0.9390	0.9161	0.8627	13.8661	<0.0001	41/04	Significant

pH (y_1), density (y_2), percent transmittance (y_3), particle size (y_4), and polydispersity index (y_5)

RESPONSE ANALYSIS OF VCO NANOEMULSION

Response analysis of pH, density, percent transmittance, particle size, and polydispersity index were carried out to see the effect of the concentration factors of Tween 80, PEG-400 and the interaction of the two factors. The effect of Tween 80 (x_1), PEG-400 (x_2), and their interaction (x_1x_2) on the response can be described by the following equation.

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2$$

where y is the response; b is the coefficient; and x is the factor.

The results of the response analysis can be seen in Table 4. Factors that influence the response are indicated by p-value <0.05. All factors, either tween 80, PEG-400 or the interaction of the two factors, significantly affect the response. The influence can be positive or negative, as seen from the notation coefficient (+) or (-). If the coefficient has a notation (+), the effect is directly proportional, where the higher the concentration used, the greater the response value. If the coefficient has a notation (-), the effect is inversely proportional; the higher the concentration, the smaller the response value (Apriani, Miksusanti & Fransiska 2022). The influence value can be seen from the value of the % contribution of each factor to the response. Tween 80 contributes the highest percentage to the response of density, percent transmittance, particle size, and PDI. PEG-400 gives a significant percent contribution to the percent transmittance and particle size. At the same time, the interaction of the two factors gave the highest percentage contribution to the pH response. Tween 80 and PEG-400 contain hydroxyl groups in their structure, so when they are used at a high concentration, the pH of the nanoemulsion preparation will increase (Rowe, Sheskey & Quinn 2009). In density response, the concentration of Tween 80 used at 40-50% will significantly affect the density of nanoemulsions. Tween 80 has a density of 1.08 g/mL (Rowe, Sheskey & Quinn 2009). The percent contribution of the two factors and their interactions are almost the same in the percent transmittance response. Tween 80 and PEG-400 have a positive effect, while the interaction of the two factors has a negative effect. The percent transmittance value close to 100% indicates that the ratio of oil in a composition smaller than the surfactant and cosurfactant composition has good clarity (Kotta et al. 2015). The clarity of nanoemulsion can be used as an indicator to predict the particle size of nanoemulsion. The higher the percentage of transmittance, the smaller the particle size of the nanoemulsion. Tween 80 and PEG-400 have a negative effect on the particle size response, while the interaction of the two factors has a positive effect. Tween 80 and PEG-400 will adsorb the surface of the nanoemulsion, so the surface tension of the two phases will decrease, which causes the kinetic stability of the emulsion system to increase (Sarheed, Dibi & Ramesh 2020). Tween 80 and PEG-400 will prevent oil droplets from getting close to each other so it can prevent aggregation. However, when the concentrations of Tween 80 and PEG 400 used are both high, it will cause the particle size to become big size. Increasing the concentration of surfactants and co-surfactants will form micelles in a continuous phase which results in the local osmotic pressure increase, so the droplets move closer to each other, causing aggregation (Hasani, Pezeshki & Hamishehkar 2015; Wu et al. 2020). The larger the particle size of the nanoemulsion, the more non-uniform the resulting droplets (PDI value > 0.5).

TABLE 4. The results of response analysis

Response	Parameter	b_0	x_1	x_2	x_1x_2
y_1	Coefficient	6.4017	0.1250	0.0850	0.1317
	p-value		< 0.0001	< 0.0001	< 0.0001
	% Contributions		38.8065	17.9441	43.0562
y_2	Coefficient	1.0643	0.0079	0.0051	-0.0016
	p-value		< 0.0001	< 0.0001	0.0006
	% Contributions		68.3557	28.1830	2.7342
y_3	Coefficient	73.6333	24.6833	24.2500	-24.1000
	p-value		< 0.0001	< 0.0001	< 0.0001
	% Contributions		34.2642	33.0717	32.6639
y_4	Coefficient	59.2208	-45.3408	-44.9292	44.8425
	p-value		< 0.0001	< 0.0001	< 0.0001
	% Contributions		33.7806	33.1699	33.0421
y_5	Coefficient	0.1184	0.0313	-0.0214	-0.0266
	p-value		< 0.0001	0.0009	0.0002
	% Contributions		42.8115	20.1078	30.9798

Tween 80 (x_1), PEG-400 (x_2), Tween 80 and PEG-400 interactions (x_1x_2), pH (y_1), density (y_2), percent transmittance (y_3), particle size (y_4), and polydispersity index (y_5), p-value <0.05, indicating a significant factor in the response

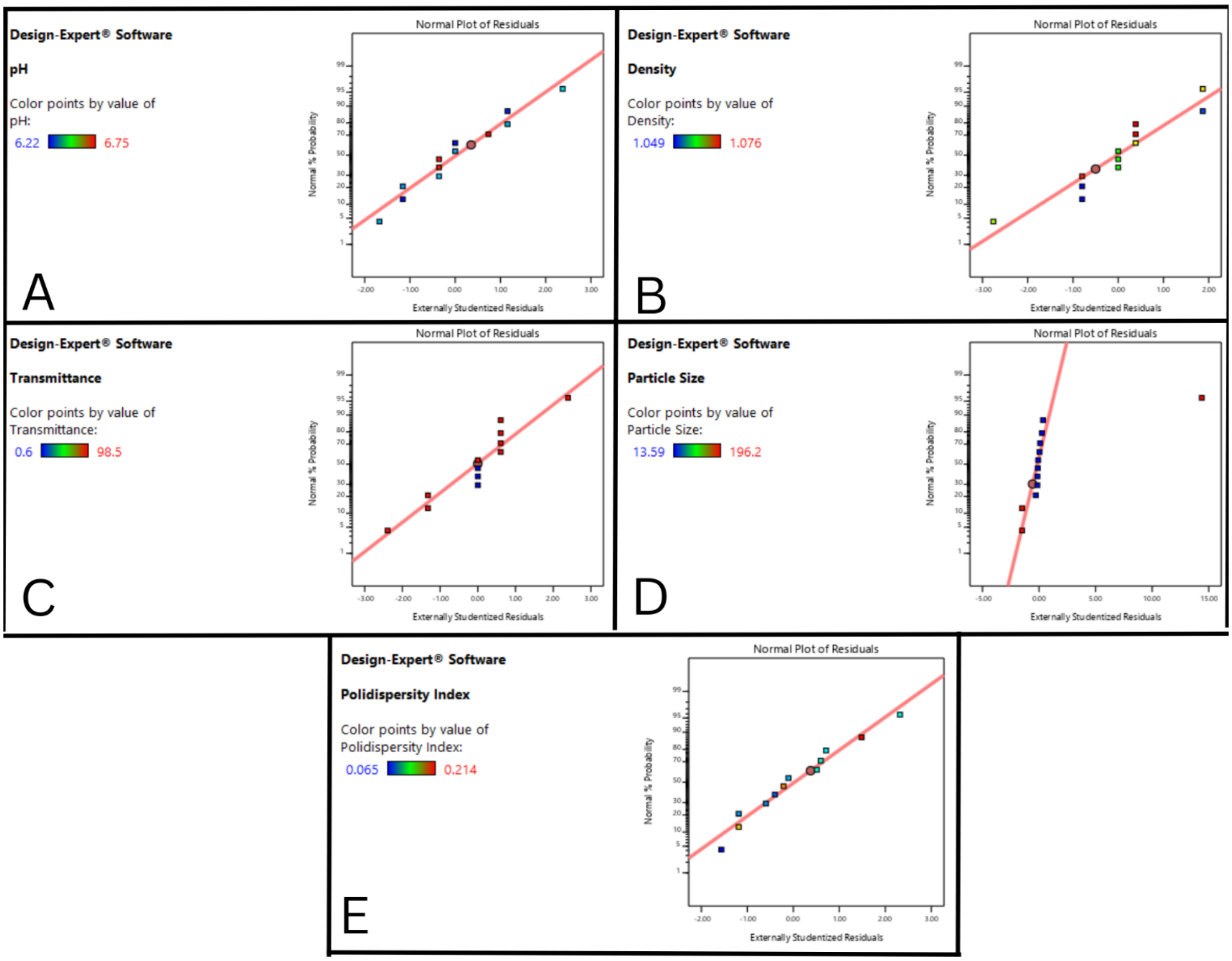

FIGURE 4. Normal Plot of residual graph on pH (A), density (B), percent transmittance (C), particle size (D), and polydispersity index (E)

The Interaction Curves can describe the interaction between Tween 80 and PEG-400 in Figure 5. Based on the interaction curve, Tween 80 and PEG-400 interact on pH response, percent transmittance, particle size, and polydispersity index, which are indicated by the intersection between the two-factor lines. Meanwhile, there is no interaction in the density response, characterised by the absence of line intersections and evidenced by the small % contribution at x_1x_2 of 2.7342%.

To predict the response value can also use the response equation obtained based on the influence of factors and factor interactions. The response equation is obtained from the coefficient values in Table 4. The response equations generated in this study are as follows: $y_1 = 6.4017 + 0.1250x_1 + 0.0850x_2 + 0.1317x_1x_2$, $y_2 = 1.0643 + 0.0079x_1 + 0{,}0051x_2 – 0.0016x_1x_2$, $y_3 = 73.6333 + 24.6833x_1 + 24.3500x_2 – 24.1000x_1x_2$, $y_4 = 59.2208 – 45.3408x_1 – 44.9292x_2 + 44.8425x_1x_2$, and $y_5 = 0.1184 + 0.0313x_1 - 0{,}0214x_2 – 0{,}0266x_1x_2$.

NANOEMULSION OPTIMUM FORMULA

The optimum formula is determined based on pH values ranging from 4.5 to 6.5, low specific gravity, high transmittance percentage, small particle size, and small PDI values. The optimum formula is selected by the Design Expert 13® system based on the desirability value of the desired goals. The concentration of the formula with the desirability value getting closer to 1 indicates that the formula is getting closer to the desired goals (Boby 2013). The optimum concentrations in this study for Tween 80 and PEG-400 were 40% and 26%, respectively, with a desirability value of 0.961. These results can be seen in the graph overlay plot in Figure 6.

STABILITY OF OPTIMUM FORMULA

This study tested the stability of the nanoemulsion based on the effect of temperature and speed. The result can be seen in Table 5. It proves that using optimum concentrations of Tween 80 and PEG-400 can increase the stability of the nanoemulsion. The nanoemulsion is stable because there is no organoleptic change (the colour is yellow and clear), no phase separation occurs, and the decrease in pH is not significant from 6.23 ± 0.01 to 6.21 ± 0.01 ($p>0.05$).

ANTIBACTERIAL ACTIVITY

The antibacterial activity of VCO nanoemulsion against *S. aureus* bacteria was described by the value of the diameter of the inhibition zone, the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC). The results of the diameter of the inhibition zone can be seen in Table 6, while the MIC and MBC can be seen in Table 7. The diameter of the inhibition zone shows that the optimum nanoemulsion formula has the largest inhibition zone diameter of 24.77 ± 1.66 mm and is included in the potent category. MIC and MBC of nanoemulsions were 1250 µg/mL and 2500 µg/mL, respectively, while the VCO values were 2500 µg/mL and more than 2500 µg/mL, respectively. So, the antibacterial activity of nanoemulsions is better than VCO without nanoemulsions. The development of nanoemulsion dosage forms can increase the antibacterial activity of VCO. The optimum nanoemulsion formula has a small particle size of 14.79±0.09 nm. Small particle size will make the surface area larger, so more particles will contact with bacteria. These particles will penetrate bacterial cells and cause the bacteria to lysis (Álvarez-Chimal et al. 2022; Cecchini et al. 2021; Moghimi et al. 2016). VCO has antibacterial activity due to the content of fatty acids such as lauric acid, palmitic acid, caprylic acid, oleic acid, capric acid, and stearic acid. These fatty acids can act as antibacterial by damaging bacterial cell membranes, interfering with

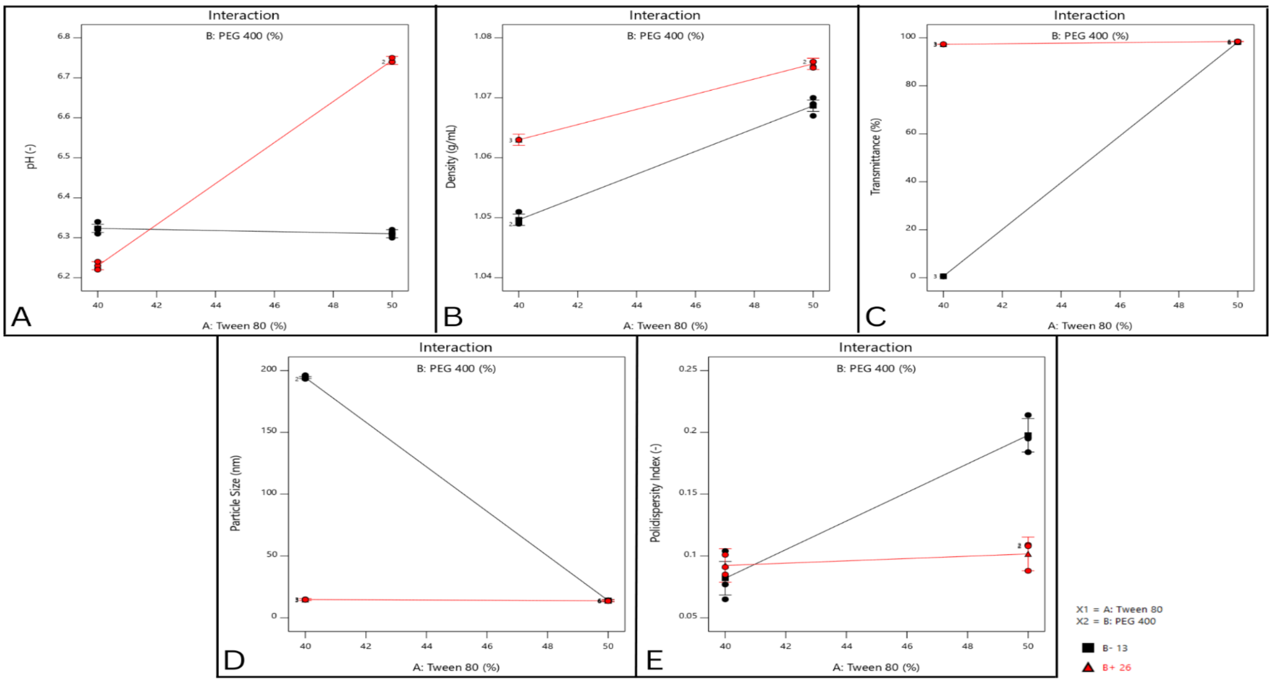

FIGURE 5. Interaction graph of pH (A), density (B), percent transmittance (C), particle size (D), and polydispersity index (E)

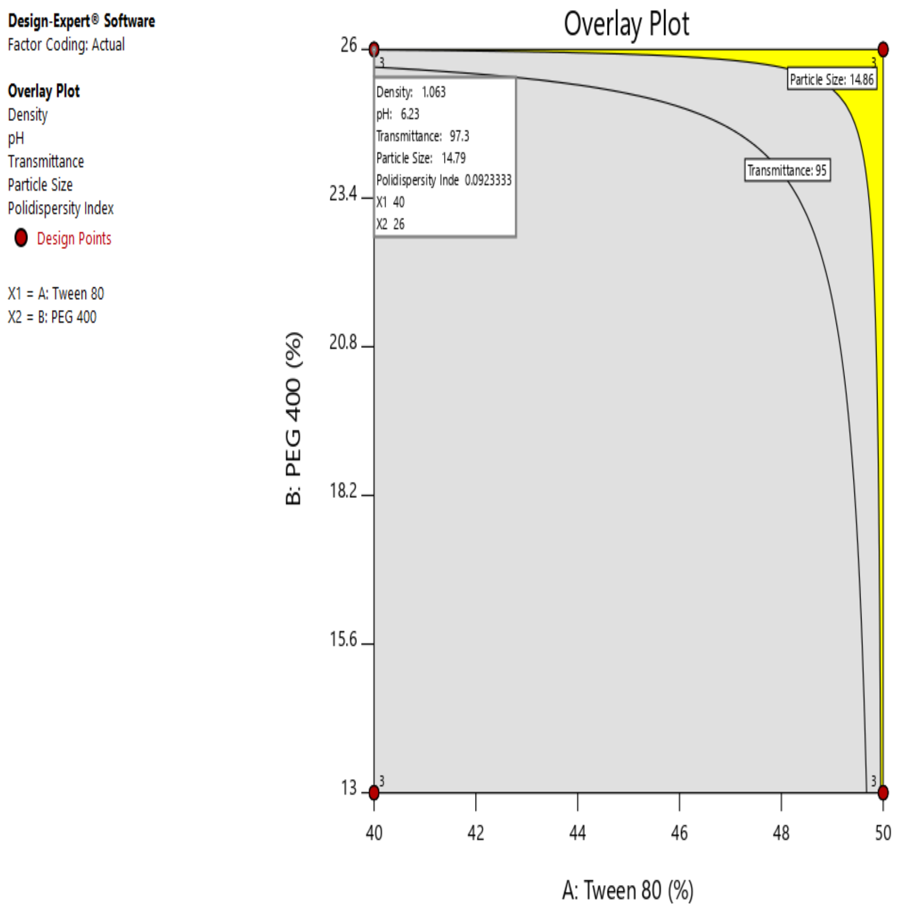

FIGURE 6. Overlay plot of optimum formula

TABLE 5. Result of stability testing

Parameter	Result	
	Before	After
Organoleptic	Pale light yellow	Pale light yellow
Phase separation	No	No
pH	6.23 ± 0.01	6.21 ± 0.01

electron transport and oxidative phosphorylation (Anzaku et al. 2017; Desbois & Smith 2010; Huang, George & Ebersole 2010; Matsue et al. 2019). The antibacterial activity of the nanoemulsion was significantly different from the positive control of erythromycin, where the nanoemulsion had better activity. Based on these results, the VCO nanoemulsion has the potential to be developed into antibacterial preparations and overcome the problem of antibiotic resistance.

TABLE 6. Results of inhibitory zone diameter

Treatment	Diameter of the inhibition zone (mm)	Category
Optimum formula	24.77 ± 1.66^{a}	Potent
VCO	13.07 ± 1.80^{c}	Strong
Positive control	19.67 ± 0.58^{b}	Strong
Negative control	0.00 ± 0.00^{d}	No

The same letter (alphabetic) in diameter of the inhibition zone showed no significantly different

TABLE 7. The result of MIC and MBC

Concentration (µg/mL)	MIC		MBC	
	Nanoemulsion	VCO	Nanoemulsion	VCO
78.13	+	+	+	+
156.25	+	+	+	+
312.50	+	+	+	+
625	+	+	+	+
1250	-	+	+	+
2500	-	-	-	+

Note: (+) indicates the presence of bacterial growth, and (-) indicates the absence of bacterial growth

CONCLUSIONS

The concentrations of Tween 80 and PEG-400 were shown to affect the characteristics of the nanoemulsion, such as pH, density, percent transmittance, particle size, and polydispersity index. The optimum concentrations of Tween 80 and PEG-400 resulting from the optimization process were 40% and 26%, respectively. The optimum formula of nanoemulsion has good physical stability where there is no phase separation and the change in pH is not significant ($p>0.05$). The antibacterial activity of the VCO nanoemulsion optimum formula was better than VCO and erythromycin, where the difference was significant ($p<0.05$). VCO nanoemulsion has antibacterial activity with potent category.

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REFERENCES

Abllah, Z. & Shahdan, I. 2018. Virgin coconut oil and its antimicrobial properties against pathogenic microorganisms: A review. *Journal of Research in Health Sciences* 8: 192-199. https://doi.org/10.2991/IDCSU-17.2018.51

Agarwal, R. 2017. Extraction processes of virgin coconut oil. *Food Processing & Technology* 4: 1-3. https://doi.org/10.15406/mojfpt.2017.04.00087

Álvarez-Chimal, R., García-Pérez, V.I., Álvarez-Pérez, M.A., Tavera-Hernández, R., Reyes-Carmona, L., Martínez-Hernández, K. & Arenas-Alatorre, J.A. 2022. Influence of the particle size on the antibacterial activity of green synthesized zinc oxide nanoparticles using *Dysphania ambrosioides* extract, supported by molecular docking analysis. *Arabian Journal of Chemistry* 15(6): 10384. https://doi.org/10.1016/j.arabjc.2022.103804

Anzaku, A.A., Akyala, A., Adeola, J. & Ewenighi, C. 2017. Antibacterial activity of lauric acid on some selected clinical isolates. *Annals of Clinical and Laboratory Research* 5: 1-5. https://doi.org/10.21767/2386-5180.1000170

Apriani, E.F., Miksusanti, M. & Fransiska, N. 2022. Formulation and optimization peel-off gel mask with polyvinyl alcohol and gelatin based using factorial design from banana peel flour (*Musa paradisiaca* L) as antioxidant. *Indonesian Journal of Pharmacy* 33(2): 261-268. https://doi.org/10.22146/ijp.3408

Apriani, E.F., Mardiyanto, M. & Hendrawan, A. 2022. Optimization of green synthesis of silver nanoparticles from *Areca catechu* L. seed extract with variations of silver nitrate and extract concentrations using simplex lattice design method. *Farmacia* 70(5): 917-924. https://doi.org/10.31925/farmacia.2022.5.18

Aziz, A. & Aziz, S. 2018. Application of box behnken design to optimize the parameters for kenaf-epoxy as noise absorber. *IOP Conference Series: Materials Science and Engineering* 454: 1-10. https://doi.org/10.1088/1757-899X/454/1/012001

Azmi, N.A.N., Elgharbawy, A.A.M., Motlagh, S.R., Samsudin, N. & Salleh, H.M. 2019. Nanoemulsions: Factory for food, pharmaceutical and cosmetics. *Processes* 7(617): 1-34. https://doi.org/10.3390/pr7090617

Boateng, L., Ansong, R., Owusu, W.B. & Steiner-Asiedu, M. 2016. Coconut oil and palm oil's role in nutrition, health and national development: A review. *Ghana Med. J.* 50(3): 189-196.

Boby, J. 2013. Application of desirability function for optimizing the performance characteristics of carbonitrided bushes. *International Journal of Industrial Engineering Computations* 4: 305-314. https://doi.org/10.5267/j.ijiec.2013.04.003

Cecchini, M.E., Paoloni, C., Campra, N., Picco, N., Grosso, M.C., Soriano-Perez, M.L., Alustiza, F., Cariddi, N. & Bellingeri, R. 2021. Nanoemulsion of *Minthostachys verticillata* essential oil. *In-vitro* evaluation of its antibacterial activity. *Heliyon* 7(1): e05896. https://doi.org/10.1016/j.heliyon.2021.e05896

Chen, Y.S., Chiu, Y.H., Li, Y.S., Lin, E.Y., Hsieh, D.K., Lee, C.H., Huang, M.H., Chuang, H.M., Lin, S.Z., Harn, H.J. & Chiou, T.W. 2019. Integration of PEG 400 into a self-nanoemulsifying drug delivery system improves drug loading capacity and nasal mucosa permeability and prolongs the survival of rats with malignant brain tumors. *Int. J. Nanomedicine* 14: 3601-3613. https://doi.org/10.2147/IJN.S193617

Chuacharoen, T., Prasongsuk, S. & Sabliov, C.M. 2019. Effect of surfactant concentrations on physicochemical properties and functionality of curcumin nanoemulsions under conditions relevant to commercial utilization. *Molecules* 24(2744): 1-12. https://doi.org/10.3390/molecules24152744

Clark, S.B. & Hicks, M.A. 2022. *Staphylococcal Pneumonia*. In StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Jan-. https://www.ncbi.nlm.nih.gov/books/NBK559152/

Desbois, A.P. & Smith, V.J. 2010. Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Appl. Microbiol. Biotechnol.* 85: 1629-1642. https://doi.org/10.1007/s00253-009-2355-3

Feßler, A.T., Li, J., Kadlec, K., Wang, Y. & Schwarz, S. 2008. Chapter 4 - Antimicrobial resistance properties of *Staphylococcus aureus.* In *Staphylococcus aureus*, edited by Fetsch, A. Massachusetts: Academic Press. pp. 57-85. https://doi.org/10.1016/B978-0-12-809671-0.00004-8.

Figueiredo, D., Junior, S. & Rocha, E. 2011. What is R2 all about? *Leviathan-Cadernos de Pesquisa Polútica* 3: 60-68. https://doi.org/10.11606/issn.2237-4485.lev.2011.132282

Fischer, C.L., Drake, D.R., Dawson, D.V., Blanchette, D.R., Brogden, K.A. & Wertz, P.W. 2012. Antibacterial activity of sphingoid bases and fatty acids against Gram-positive and Gram-negative bacteria. *Antimicrob Agents Chemother.* 56(3): 1157-1161. https://doi.org/10.1128/AAC.05151-11

Gupta, A., Eral, H.B., Hatton, T.A. & Doyle, P.S. 2016. Nanoemulsions: Formation, properties and applications. *Soft Matter.* 12: 2826-2841. https://doi.org/10.1039/C5SM02958A

Hasani, F., Pezeshki, A. & Hamishehkar, H. 2015. Effect of surfactant and oil type on size droplets of betacarotene-bearing nanoemulsions. *Int. J. Curr. Microbiol. App. Sci.* 4: 146-155.

Homayoonfal, M., Khodaiyan, F. & Mousavi, S.M. 2014. Walnut oil nanoemulsion: Optimization of the emulsion capacity, cloudiness, density, and surface tension. *Journal of Dispersion Science and Technology* 35(5): 725-733. https://doi.org/10.1080/01932691.2013.807742

Huang, C.B., George, B. & Ebersole, J.L. 2010. Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. *Arch. Oral Biol.* 55(8): 555-560. https://doi.org/10.1016/j.archoralbio.2010.05.009

Kayili, E. & Sanlibaba, P. 2020. Prevalence, characterization and antibiotic resistance of *Staphylococcus aureus* isolated from traditional cheeses in Turkey. *International Journal of Food Properties* 23(1): 1441-1451. https://doi.org/10.1080/10942912.2020.1814323

Kenechukwu, O., Chukwuemeka, O., Joy, I. & Edwin, E. 2022. Effect of virgin coconut oil, lauric acid and myristic acid on serum and prostatic markers of benign prostatic hyperplasia. *Tropical Journal of Pharmaceutical Research* 21: 809-815. https://doi.org/10.4314/tjpr.v21i4.18

Kotta, S., Khan, A.W., Ansari, S.H., Sharma, R.K. & Ali, J. 2015. Formulation of nanoemulsion: A comparison between phase inversion composition method and high-pressure homogenization method. *Drug Delivery* 22(4): 455-466. https://doi.org/10.3109/10717544.2013.866992

Kumar, S., Singh, S., Kumar, V., Datta, S., Sharma, P. & Singh, J. 2020. Pathogenesis and antibiotic resistance of *Staphylococcus aureus*. In *Model Organisms for Microbial Pathogenesis, Biofilm Formation and Antimicrobial Drug Discovery*, edited by Siddhardha, B., Dyavaiah, M. & Syed, A. Singapore: Springer. pp. 99-115. https://doi.org/10.1007/978-981-15-1695-5_7

Laxmi, M., Bhardwaj, A., Mehta, S. & Mehta, A. 2015. Development and characterization of nanoemulsion as carrier for the enhancement of bioavailability of artemether. *Artificial Cells, Nanomedicine, and Biotechnology* 43(5): 334-344. https://doi.org/10.3109/21691401.2014.887018

Liu, Q., Huang, H., Chen, H., Lin, J. & Wang, Q. 2019. Food-grade nanoemulsions: Preparation, stability and application in encapsulation of bioactive compounds. *Molecules* 24(23): 4242. https://doi.org/10.3390/molecules24234242

Lu, H. & Tan, P.P. 2009. A comparative study of storage stability in virgin coconut oil and extra virgin olive oil upon thermal treatment. *International Food Research Journal* 16: 343-354.

Man, G., Elias, P.M. & Man, M.Q. 2015. Therapeutic benefits of enhancing permeability barrier for atopic eczema. *Dermatologica Sinica* 33(2): 84-89. https://doi.org/10.1016/j.dsi.2015.03.006

Matsue, M., Mori, Y., Nagase, S., Sugiyama, Y., Hirano, R., Ogai, K., Ogura, K., Kurihara, S. & Okamoto, S. 2019. Measuring the antimicrobial activity of lauric acid against various bacteria in human gut microbiota using a new method. *Cell Transplant* 28(12): 1528-1541. https://doi.org/ 10.1177/0963689719881366

Mardiyanto, M., Apriani, E.F. & Alfarizi, M.H. 2022. Formulation and *in-vitro* antibacterial activity of gel containing ethanolic extract of purple sweet potato leaves (*Ipomoea batatas* (L.) loaded poly lactic co-glycolic acid submicroparticles against *Staphylococcus aureus*. *Research Journal of Pharmacy and Technology* 15(8): 3599-5. https://doi.org/10.52711/0974-360X.2022.00603

Moghimi, R., Aliahmadi, A., McClements, D.J. & Rafati, H. 2016. Investigations of the effectiveness of nanoemulsions from sage oil as antibacterial agents on some food borne pathogens. *LWT - Food Science and Technology* 71: 69-76. https://doi.org/10.1016/j.lwt.2016.03.018

Nakatsuji, T., Kao, M., Fang, J.Y., Zouboulis, C., Zhang, L., Gallo, R. & Huang, C.M. 2009. Antimicrobial property of lauric acid against *Propionibacterium acnes*: Its therapeutic potential for inflammatory acne vulgaris. *The Journal of Investigative Dermatology* 129: 2480-2488. https://doi.org/10.1038/jid.2009.93

Nielsen, C.K., Kjems, J., Mygind, T., Snabe, T. & Meyer, R.L. 2016. Effects of Tween 80 on growth and biofilm formation in laboratory media. *Front. Microbiol.* 7(1878): 1-10. https://doi.org/10.3389/fmicb.2016.01878

Noordin, M.Y., Venkatesh, V.C., Sharif, S., Elting, S. & Abdullah, A. 2004. Application of response surface methodology in describing the performance of coated carbide tools when turning AISI 1045 steel. *Journal of Materials Processing Technology* 15(1): 46-58. https://doi.org/10.1016/S0924-0136(03)00861-6

Nurhidayah, E., Agustin, A., Indawati, I., Zamzam, M.Y. & Nabila, S.P. 2022. The characteristics of virgin coconut oil made in oil fishing method and gradual heating. *Jurnal Kesehatan Muhamadiyah* 3(1): 35-40. https://doi.org/10.37874/mh.v3i1.400

Parker, D. & Prince, A. 2012. Immunopathogenesis of *Staphylococcus aureus* pulmonary infection. *Semin Immunopathol.* 34(2): 281-297. https://doi.org/10.1007/s00281-011-0291-7

Rowe, R.C., Sheskey, P.J. & Quinn, M.E. 2009. *Handbook of Pharmaceutical Excipients*. 6th ed. London: Pharmaceutical Press.

Rukmini, A. & Raharjo, S. 2010. Pattern of peroxide value changes in virgin coconut oil (VCO) due to photo-oxidation sensitized by chlorophyll. *Journal of the American Oil Chemists' Society* 87: 1407-1412. https://doi.org/10.1007/s11746-010-1641-7

Sarheed, O., Dibi, M. & Ramesh, K.V.R.N.S. 2020. Studies on the effect of oil and surfactant on the formation of alginate-based O/W lidocaine nanocarriers using nanoemulsion template. *Pharmaceutics* 12(12): 1223. https://doi.org/10.3390/pharmaceutics12121223

Shehata, T.M., Almostafa, M.M. & Elsewedy, H.S. 2022. Development and optimization of *Nigella sativa* nanoemulsion loaded with pioglitazone for hypoglycemic effect. *Polymers* 14: 3021. https://doi.org/10.3390/polym14153021

Sivakanthan, S., Bopitiya, D. & Madhujith, T. 2018. A comparative study on stability of different types of coconut (*Cocos nucifera*) oil against autoxidation and photo-oxidation. *African Journal of Food Science* 12(9): 216-229. https://doi.org/10.5897/AJFS2018.1695

Su'i, M., Sumaryati, E., Prasetyo, R. & Eric, P. 2015. Anti-bacteria activities of lauric acid from coconut endosperm (Hydolysed using lipase Endogeneus). *Advance in Environmental Biology* 9(23): 45-49.

Suryani, S., Sariani, S., Earnestly, F., Marganof, M., Rahmawati, R., Sevindrajuta, S., Mahlia, T.M.I. & Fudholi, A. 2020. A comparative study of virgin coconut oil, coconut oil and palm oil in terms of their active ingredients. *Processes* 8(402): 1-11. https://doi.org/10.3390/pr8040402

Standar Nasional Indonesia. 2008. *SNI 7381:2008 Virgin Coconut Oil*. Jakarta: Badan Standardisasi Nasional. pp. 1-36.

Thakkar, H., Nangesh, J., Parmar, M. & Patel, D. 2011. Formulation and characterization of lipid-based drug delivery system of raloxifene microemulsion and self-microemulsifying drug delivery system. *J. Pharm. Bioallied. Sci.* 3(3): 442-448. https://doi.org/10.4103/0975-7406.84463

Tong, S.Y., Davis, J.S., Eichenberger, E., Holland, T.L. & Fowler Jr., V.G. 2015. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clin. Microbiol. Rev.* 28(3): 603-661. https://doi.org/10.1128/CMR.00134-14

Ujilestari, T., Dono, N., Ariyadi, B., Martien, R. & Zuprizal, Z. 2018. Formulation and characterization of self-nano emulsifying drug delivery systems of lemongrass (*Cymbopogon citratus*) essential oil. *Malaysian Journal of Fundamental and Applied Sciences* 14: 360-363. https://doi.org/10.11113/mjfas.v14n3.1070

Wu, H.R., Wang, C.Q., Wang, J.X., Chen, J.F. & Le, Y. 2020. Engineering of long-term stable transparent nanoemulsion using high-gravity rotating packed bed for oral drug delivery. *Int. J. Nanomedicine* 15: 2391-2402. https://doi.org/10.2147/IJN.S238788

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