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Original Article

NANOEMULSION OF CHAMPACA FLOWER (*MAGNOLIA ALBA*) OIL AS AN ANTIBACTERIAL CANDIDATE: OPTIMIZATION, CHARACTERIZATION, AND THERMODYNAMIC STABILITY TESTING

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ABSTRACT

Objective: This study aims to investigate the antibacterial potential of *Magnolia alba* (*M. alba*) essential oil and to optimize the composition of the oil, surfactant mix, and distilled water in order to formulate a stable topical nanoemulsion of *M. alba*. The focus is on enhancing the thermodynamic stability and achieving a small particle size of the nanoemulsions.

Methods: The chemical composition of champaca oil was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), and its antibacterial activity against *Staphylococcus aureus* was evaluated through a dilution test, with ciprofloxacin serving as the positive control. The nanoemulsion was optimized using the Simplex Lattice Design (SLD) method in Design Expert 13.0, employing Virgin Coconut Oil (VCO) as the oil phase, Tween 80 as the surfactant, PEG 400 as the co-surfactant, and distilled water as the aqueous phase. We selected the optimal formula based on pH and transmittance values. The optimized nanoemulsion was further characterized for droplet size, zeta potential, and polydispersity index and was subjected to thermodynamic stability tests.

Results: *M. alba* oil contains cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester (15.83%), benzyl alcohol (6.89%), phenyl ethyl alcohol (5.72%), and linalool (4.31%), with a Minimum Inhibitory Concentration (MIC) of 2%. The optimized nanoemulsion formulation, comprising of 4% oil phase, 27% surfactant mix (Smix), and 69% aqueous phase, was clear and stable, with a pH of 5.33, a transmittance of 98.69%, a droplet size of 30.55 nm, a zeta potential of 1.21 mV, and a polydispersity index of 0.026.

Conclusion: *M. alba* oil exhibits promising antibacterial properties against *Staphylococcus aureus. The* optimized nanoemulsion formulation achieves thermodynamic stability and small droplet size, making it a potential candidate for topical antibacterial applications. Further investigations are required to assess long-term stability.

Keywords: Antibacterial, *Magnolia alba*, Nanoemulsion, Simplex lattice design, Thermodynamic stability

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INTRODUCTION

White champaca (*Magnolia alba*) is an evergreen tropical plant known for its distinctive and fragrant aroma. In Bali, the unique scent of champaca flowers is widely utilized in religious offerings, aromatherapy, essential oils, and incense. Beyond these traditional applications, white champaca flowers exhibit potential antibacterial, antifungal, antidiabetic, anti-inflammatory, antioxidant, and antidepressant properties [1–3]. The methanol extract of *M. alba* demonstrates antibacterial activity against *Staphylococcus aureus*, showing a moderate to strong inhibitory effect. These effects are attributed to the secondary metabolites present in the extract, including alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids [2, 4, 5]. However, the use of extracts in formulations can often lead to aesthetically unappealing and unstable products, highlighting the need for alternative forms such as oils or essential oils. Almost all parts of the champaca flower can yield essential oils, with varying concentrations depending on the specific part used, typically dominated by linalool [3, 6]. Champaca oil has been shown to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* [7]. Given this potential, champaca oil can be developed into a topical preparation that serves as an anti-acne agent, thereby reducing the reliance on synthetic chemicals and antibiotics.

Since champaca oil is lipophilic, it is well-suited for formulation in oil-based preparations, such as emulsions. Emulsions are biphasic systems in which one liquid is dispersed in another as droplets. These systems typically consist of an oil phase, a water phase, and an emulsifier. However, emulsions can encounter physical stability challenges, such as phase separation during storage [8]. Lipophilic ingredients like champaca oil can be formulated into nanoemulsions. Nanoemulsions are heterogeneous systems of immiscible liquids dispersed as droplets smaller than 100 nm. They offer a more visually appealing, transparent, and stable system with reduced separation, improved permeability of active ingredients, and enhanced solubility, thereby improving skin delivery [9, 10].

A nanoemulsion is composed of a mixture of water, oil, and a surfactant mix (Smix), which includes both a surfactant and a co-surfactant. The selection and ratio of the surfactant and co-surfactant are critical for achieving a stable nanoemulsion, often assessed using the Hydrophilic-Lipophilic Balance (HLB) value. The optimal HLB value for a stable nanoemulsion typically falls within the range of 12 to 20 [11], with a preferred surfactant-to-co-surfactant ratio of 2:1 [12]. This study aims to develop a stable nanoemulsion of champaca flower oil, employing formula optimization through Simplex Lattice Design in Design Expert software to identify the optimal proportions of the oil, Smix, and water phases.

MATERIALS AND METHODS

Materials

Essential oils of *M. alba* were purchased from Botanica Asri (Surabaya, Indonesia). Virgin coconut oil, Tween 80, PEG 400 (Bratachem, Indonesia), Aquadest, DMSO (SABA, Indonesia), NaCl 0.9% (PT. Widatra Bhakti, Indonesia), ciprofloxacin (The Indonesia Food and Drug Authority, Indonesia), *Staphylococcus aureus* bacterial suspension (Balai Laboratorium Kesehatan Kerthi Bali Sadhajiwa, Indonesia), and Mueller Hinton broth were also obtained.

Characterization of essential oils

The characterization of essential oils encompasses several analytical tests, including organoleptic assessment, refractive index measurement, solubility evaluation, chemical composition analysis, and antibacterial activity determination. The organoleptic assessment involved the evaluation of the consistency, fragrance, and color of the essential oils. The refractive index was measured using an Abbe refractometer, with 3-4 drops of essential oil applied to the prism surface at room temperature. For the solubility evaluation, 1 ml of essential oil was combined with 96% ethanol, with the ethanol being added dropwise until a clear solution was obtained. The chemical composition of the essential oil was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) with an Agilent 8860 GC and 5977B GC/MSD, equipped with an HP-5MS UI column (30 m x 0.25 mm x 0.25 µm). The temperature program was set to increase from 70 °C to 290 °C at a rate of 10 °C per minute [3].

The antibacterial activity assay was conducted to ascertain the Minimum Inhibitory Concentration (MIC) of the essential oil against *Staphylococcus aureus*. The methodology was adapted by the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [13, 14]. The essential oil was diluted in 10% dimethyl sulfoxide (DMSO) to create five varying concentrations of 1%, 2%, 3%, 4%, and 5%. Each concentration (1 ml) was combined with 1 ml of Mueller Hinton Broth (MHB) and 1 ml of bacterial suspension. The resulting mixtures were incubated for a duration of 18 to 24 h at 37 °C in an incubator [15]. The optical density of each sample was measured using a McFarland densitometer. The negative control comprised MHB with bacterial suspension, while the positive control utilized 20% ciprofloxacin and the background control consisted solely of MHB. The MIC is defined as the lowest concentration that inhibits bacterial growth by more than 80%. The percentage of growth in each sample was calculated as follows: [16]

$$\frac{\text{ODsample} - \text{ODbackground}}{\text{ODnegative control} - \text{ODbackground}} \text{ x } 100\%$$

Optimization of nanoemulsion

The compositions of virgin coconut oil (VCO), essential oil, surfactant mix (smix), and water were formulated utilizing Simplex Lattice Design (SLD) in Design-Expert version 13. The ratio of surfactant to co-surfactant was maintained at 2:1, resulting in a combined Hydrophilic-Lipophilic Balance (HLB) value of 14.37. According to the literature, the recommended HLB value for the formulation of stable nanoemulsions typically falls within the range of 12 to 20 [17]. The upper limits for the oil, surfactant mixture, and water were established at 5%, 28%, and 71%, respectively, while the lower limits were set at 3%, 26%, and 69%. The optimization results demonstrated that the ideal nanoemulsion formulation achieved a pH level suitable for skin application and exhibited a high degree of clarity. The optimal formulation will subsequently undergo characterization tests for nanoemulsions, which will include assessments of clarity, droplet size, polydispersity index, and zeta potential.

Nanoemulsion preparations

The nanoemulsion was prepared using a combination of virgin coconut oil (VCO), essential oil from *M. alba*, Tween 80 as a surfactant, polyethylene glycol (PEG) 400 as a co-surfactant, and distilled water. The oil phase and surfactant mixture were homogenized using a magnetic stirrer at 500 rpm for 30 min to form a self-nano-emulsifying drug delivery system (SNEDDS). The resulting mixture was then subjected to sonication at 55 °C for 10 min. Subsequently, the water phase (distilled water) was added dropwise at the same temperature, followed by an additional mixing period of 30 min to facilitate the formation of the nanoemulsion. The nanoemulsion was sonicated again for 10 min to enhance stability and achieve smaller droplet sizes. The mixture is store for 24 h to ensure complete solubilization and miscibility of the essential oil.

Physical evaluation, characterization, and thermodynamic stability of nanoemulsion

The physical evaluation of the nanoemulsion encompassed organoleptic assessments, pH measurements, and viscosity tests. The organoleptic evaluation examined the consistency, fragrance, and color of the nanoemulsion. pH measurements were conducted using a calibrated pH meter (Ohaus, USA) with buffer solutions of pH 4.00 and 7.00, performed in triplicate and without dilution. The acceptable pH range for the nanoemulsion is established between 4.5 and 6.5. Viscosity was assessed using a Brookfield viscometer at a rotational speed of 100 rpm, with torque values varying between 10% and 100%.

The selected optimal formula derived from the SLD analysis will undergo characterization tests for the nanoemulsion, which will include assessments of clarity, droplet size, polydispersity index, and zeta potential. Clarity will be evaluated by measuring the percentage of transmittance using UV-Spectrophotometry (Shimadzu UV-1800, Japan) at a wavelength of 650 nm, with distilled water serving as the blank and without any dilution. An optimal clarity for the nanoemulsion is defined as a transmittance value exceeding 95% or approaching 100%, which indicates that the formulation is clear and transparent [9, 18]. The droplet size, polydispersity index, and zeta potential will be determined using a Particle Size Analyzer (PSA) (Horiba, Japan) with ten-fold dilutions.

Thermodynamic stability tests were conducted to evaluate the stability of the nanoemulsion under extreme temperatures and centrifugal forces. The assessment of thermodynamic stability involved a heating-cooling cycle, during which the nanoemulsion was alternately stored at 4 °C and 45 °C in a climatic chamber (Memmert, Germany) for a total of six cycles. Following each cycle, organoleptic properties, pH, and transmittance percentage were recorded. Formulations that met the specified criteria were subjected to centrifugation at 3500 rpm for 30 min, followed by a physical examination to assess the potential for separation. Formulations that exhibited no signs of separation were further subjected to a freeze-thaw test, which was conducted between-21 °C and 25 °C for three cycles, with each cycle lasting a minimum of 48 h [18].

RESULTS

Characterization of *M. alba* essential oil

Characterization tests were conducted to ascertain the specifications of the oil utilized in this study. These tests encompassed organoleptic properties, refractive index, solubility, chemical composition, and minimum inhibitory concentration (MIC), as detailed in tables 1 and 2.

Table 1: Characterization of *M. alba* essential oil

Organoleptic	Refractive index (28,1 °C)	Solubility	MIC
Consistency: liquid Fragrance: champaca flower Color: light yellow	1.449	Soluble in 2 ml of ethanol 96%	2% of essential oil with OD 0.84 and % growth of bacteria 13.551 %± 2.822

Notes: MIC = Minimum Inhibitory Concentration; OD = Optical Density

The optical density measurement indicates that a concentration of 2% essential oil can inhibit the growth of *Staphylococcus aureus* by over 80%. This finding is corroborated by the optical density value, which reflects a bacterial growth rate of 13.551% in the mixture, thereby confirming the minimum inhibitory concentration (MIC) of *M. alba* essential oil.

The chemical composition of *M. alba* essential oil, as determined by the GC-MS test results, is detailed in table 2, and the corresponding chromatogram is illustrated in fig. 1.

Optimization formula of nanoemulsion

Based on the established upper and lower limits defined in the design parameters, a total of 14 formula runs were generated using the Statistical Design of Experiments (SLD), as presented in table 3. The criteria for determining the lower and upper limits for formula optimization with SLD stipulate that the sum of the upper limit of the oil phase, the lower limit of the smix, and the lower limit of the water phase must equal the sum of the lower limit of the oil phase, the upper limit of the smix, and the lower limit of the water phase. This relationship is also equivalent to the sum of the lower limit of the oil phase, the lower limit of the smix, and the upper limit of the water phase, which collectively totals 100% [11]. It is noteworthy that there are four pairs of formulas exhibiting identical proportions, particularly when the number of components is minimal. This repetition is an intentional aspect of the design employed to assess the variability and reproducibility of the formulation process. Additionally, table 3 includes the pH and transmittance percentage for each run, which were designated as output variables within the SLD program.

Table 2: Chemical composition of *M. alba* essential oil

Retention time	Chemical composition	Area (%)
3.870	Beta-pinene	0.09
3.922	Beta-myrcene	0.16
4.402	D-Limonene	1.24
4.537	Benzyl alcohol	6.89
5.307	Linalool	4.31
5.590	Phenylethyl alcohol	5.72
5.964	(+)-2-Bornanone	0.05
6.499	Benzenemethanol, alpha-methyl-acetate	2.69
6.943	Citronellol	1.39
7.281	Linalyl acetate	3.72
7.927	Indole	0.06
8.515	2,6-octadiene, 2,6-dimethyl-	1.36
8.905	Geranyl acetate	0.12
9.551	Caryophyllene	0.04
9.855	Trans-isoeugenol	0.04
11.577	Caryophyllene oxide	0.06
12.031	Ethyl mesityl acetate	0.28
12.258	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	15.83

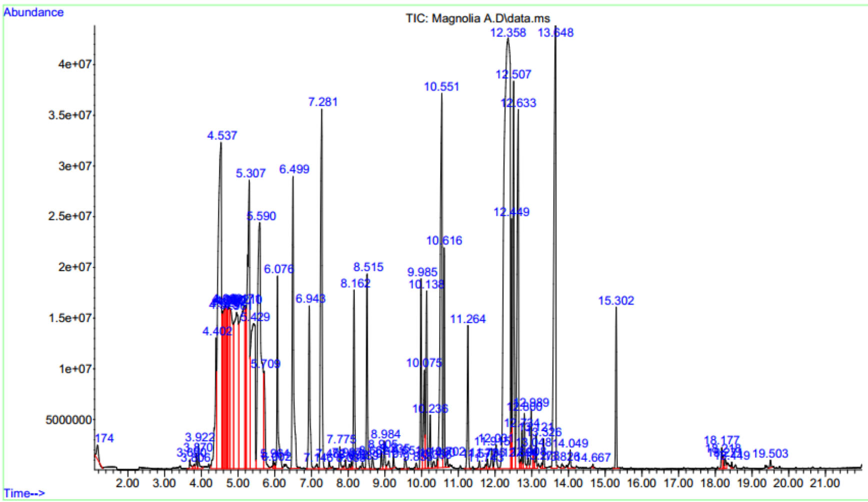


Fig. 1: Chromatogram of *M. alba* essential oil

Table 3: Optimization formula of nanoemulsion from SLD

Formula	Oil (%)	Smix (%)	Water (%)	pH[a]	Transmittance (%)[a]
1	3	26	71	5.177±0.023	98.445±2.183
2	5	26	69	5.353±0.006	97.769±1.646
3	3.33333	26.3333	70.3333	5.893±0.012	98.325±1.831
4	4.33333	26.3333	69.3333	5.1±0.017	97.718±1.978
5	4	27	69	5.093±0.012	94.206±1.751
6	3	28	69	5.090±0.066	98.496±2.507
7	5	26	69	5.123±0.068	97.917± 2.620
8	3	26	71	5.053±0.067	97.088±2.101
9	3.66667	26.6667	69.6667	5.057±0.012	99.699±0.281
10	4	26	70	5.167±0.025	98.684±0.295
11	3.33333	27.3333	69.3333	5.057±0.086	99.628±0.114
12	4	27	69	5.237±0.029	99.439±0.100
13	3	27	70	5.1±0.056	99.820±0.062
14	3	28	69	5.097±0.015	99.385±0.354

[a]Data are expressed in mean±SD (n = 3)

All formulations of the nanoemulsion met the specified criteria, exhibiting pH values between 4.5 and 6.5 and demonstrating transmittance levels nearing 100%.

Further analysis was conducted to optimize and identify the most effective formulation of the nanoemulsion. Utilizing the transmittance data presented in table 3 and the SLD obtained through Design Expert, a quadratic equation can be formulated as follows (Equation 1).

$$Y = 97.82\,(A)+98.92\,(B)+97.75\,(C) - 6.36\,(AB)+3.62\,(AC)+5.61\,(BC) - 5.58\,(A^2BC)+120.30\,(AB^2C) - 43.66\,(ABC^2) \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Notes:

Y = Transmittance

A = Oil

B = Smix

C = Water

The analysis of variance (ANOVA) conducted on the quadratic model produced a p-value greater than 0.05, indicating that there is no statistically significant difference in transmittance across the different compositions of the oil phase, surfactant mixt (smix), and water. Additionally, the lack of fit analysis yielded a p-value exceeding 0.05 at a significance level of 95%, which suggests that the model adequately fits the data without exhibiting any systematic error. The residual and contour plots for transmittance are presented in fig. 2.

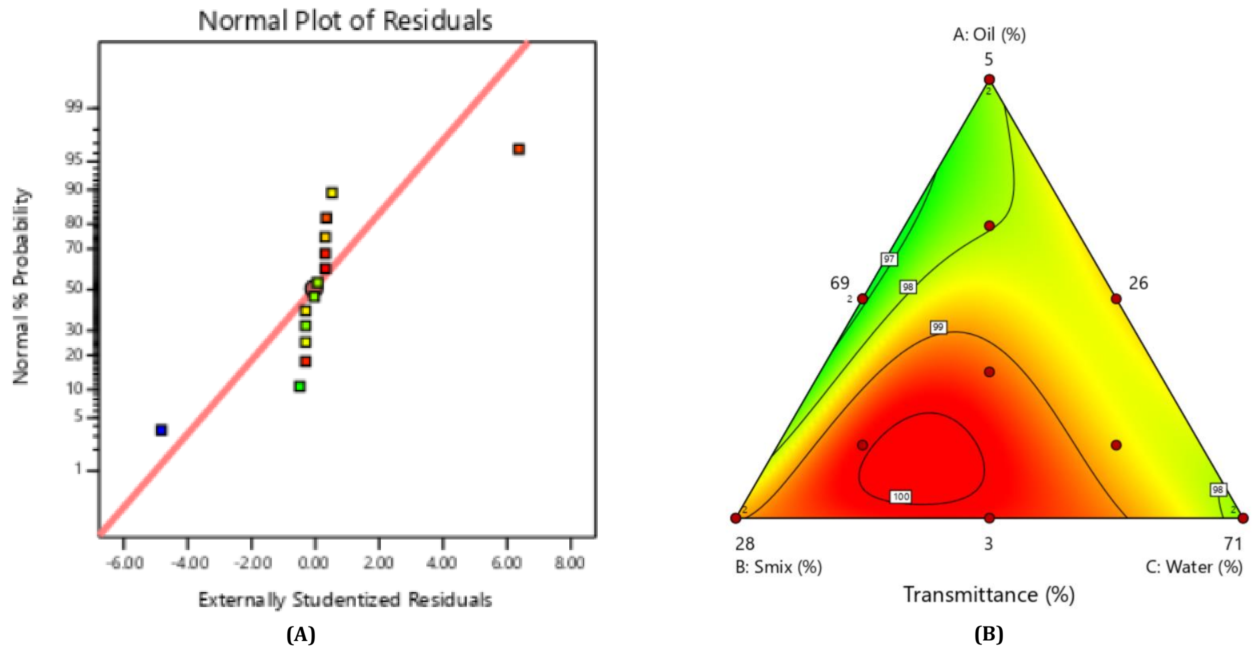


Fig. 2: (A) Normal plot of transmittance; (B) contour plot the response of transmittance

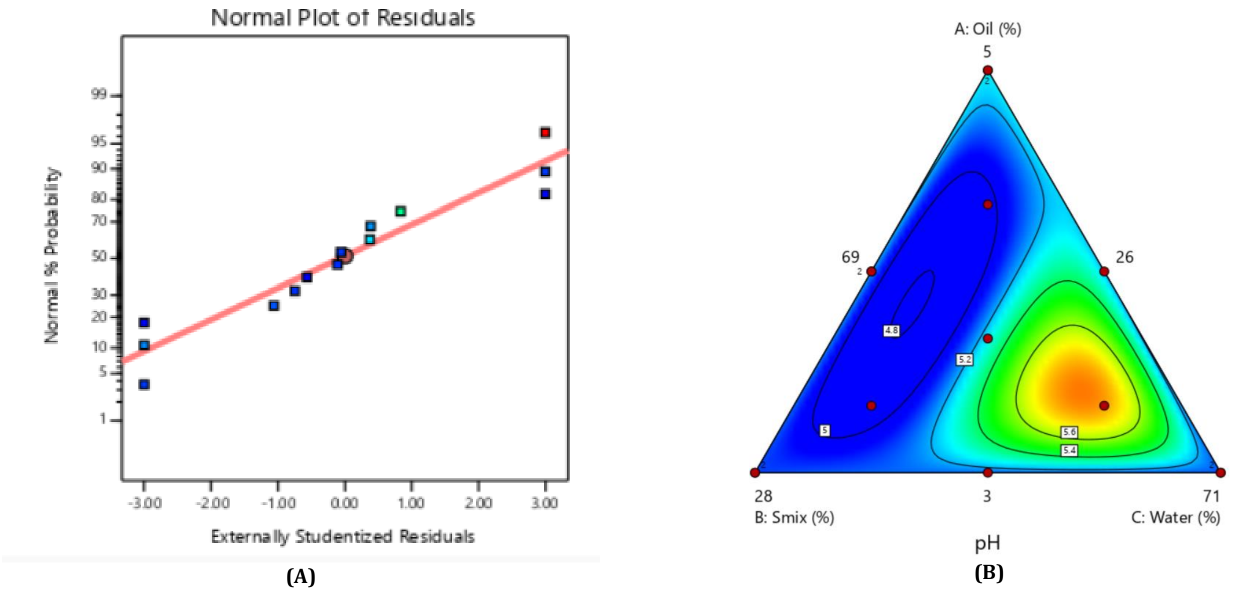


Fig. 3: (A) Normal plot of pH; (B) Contour plot illustrating the response of pH

Fig. 2 demonstrates that the green region is associated with the lowest transmittance, succeeded by the yellow and red regions. A decrease in the oil phase has impacted the red areas. Regarding the pH response, the specific quartic and quadratic equations can be expressed as follows (Equation 2).

$$*Y = 5.25 (A)+5.10 (B)+5.13 (C)+0.0417 (AB)+0.0960 (AC)+0.1170 (BC) – 29.65 (A^2BC) – 26.68 (AB^2C)+61.96 (ABC^2) \ldots\ldots\ldots (2)$$

*Y = pH

The special quartic and quadratic models demonstrated a p-value greater than 0.05, indicating no statistically significant difference in pH levels attributable to variations in the oil phase, surfactant mixture (smix), and water. However, the lack of fit analysis yielded a p-value less than 0.05, suggesting a significant discrepancy between the predicted model and the actual observations. The residual and contour plots for pH are illustrated in fig. 3.

Fig. 3 illustrates that the blue regions represent the lowest pH levels, followed by green and yellow regions. According to the analysis conducted using Design Expert, the optimal nanoemulsion formulation comprised 3.54% oil phase, 26.95% surfactant and co-surfactant mixture (smix), and 69.51% water phase, as depicted in fig. 4. This optimal formulation was subsequently compared with the predictive response derived from the SLD analysis. Verification of the results was performed using a one-sample t-test, utilizing IBM SPSS version 22, as presented in table 4.

Characterization of the optimal formula for nanoemulsion

The chosen optimal formula was re-formulated and subsequently subjected to a series of characterization tests, which included assessments of transmittance, pH, droplet size, polydispersity index, and zeta potential, as detailed in table 5 below.

Table 4: Verification of the optimal formula

Response	Predicted value	Observed value	P value
pH	5.1873	5.513	0.001
Transmittance	96.7803	99.397	0.002

The data presented indicate that the p-value for the pH response and transmittance is less than 0.05, suggesting a statistically significant difference between the predicted and observed values.

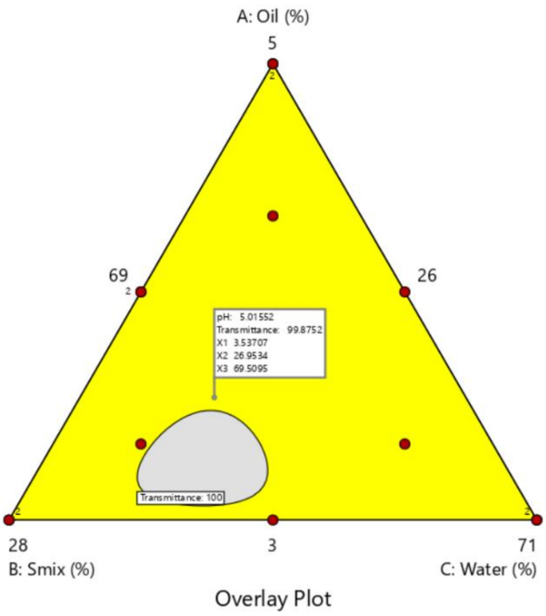


Fig. 4: The Overlay plot of the optimal formula

Table 5: Characterization of optimum formula

Transmittance (%)[a]	pH[a]	Droplet size (nm)	Polydispersity index	Zeta potential
99.397	5.513	24.1	0.107	-1.14

[a]Data are expressed in mean (n = 3)

The results presented in table 5 indicate that the optimal formulation can be classified as a nanoemulsion characterized by a droplet size of less than 100 nm and exhibiting a clear and transparent appearance, as evidenced by a high percentage of transmittance. The polydispersity index (PDI) serves as an indicator of the homogeneity of the nanoemulsion particles, with values approaching 0 signifying a more uniform droplet size distribution [18, 19]. A PDI value of less than 0.5 suggests a consistent size distribution of globules, thereby confirming the uniformity of the droplet distribution within the nanoemulsion. Furthermore, emulsifiers play a critical role in reducing the interfacial tension between water and oil while also generating zeta potential surface charges that create repulsive forces to prevent coalescence. A zeta potential value near ±30 mV is indicative of a stable colloidal system [18, 20]. However, it is important to note that zeta potential is not the sole predictor of nanoemulsion stability. In this study, the observed low zeta potential value may be attributed to the substantial quantity of non-ionic surfactants employed [21]. Nevertheless, the nanoemulsion maintained a stable appearance, as corroborated by the results of the subsequent thermodynamic stability tests.

Physical properties and thermodynamic stability of nanoemulsions

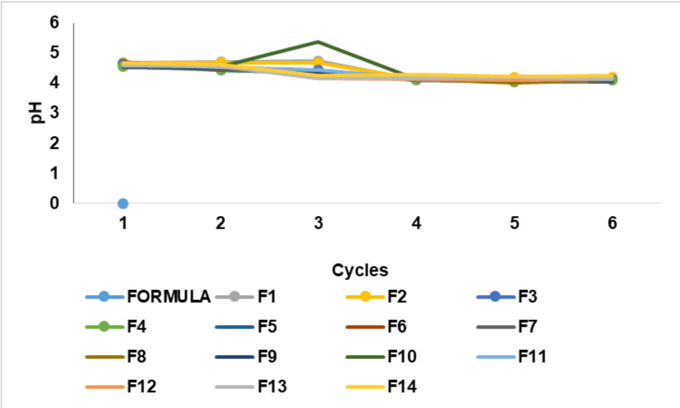
The physical evaluation of the nanoemulsion included assessments of organoleptic properties, viscosity, and stability after 24 h of formulation. All formulations displayed a liquid nanoemulsion characterized by the distinctive fragrance of champaca flowers. The parameters evaluated are presented in table 6 below.

Table 6: Physical evaluation of nanoemulsion

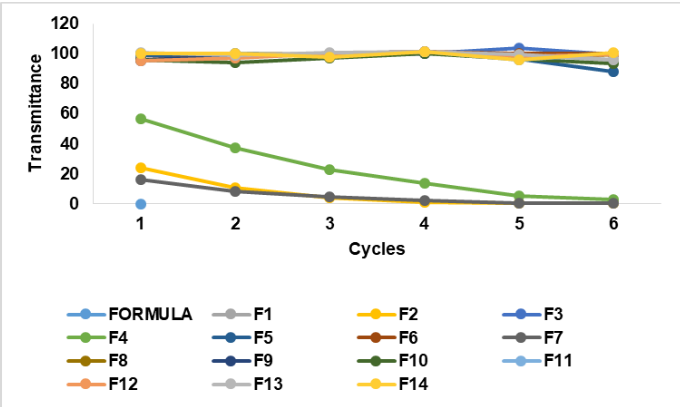
Formula	Organoleptic (color)	Viscosity (cps)[a]	Stability (24 h)
1	Transparent	6.400±0.130	Stable
2	Slightly yellow	9.300±0.035	Stable
3	Transparent	7.593±0.075	Stable
4	Slightly yellow	8.727±0.199	Stable
5	Slightly yellow	9.267±0.580	Stable
6	Transparent	8.467±0.329	Stable
7	Slightly yellow	7.767±0.131	Stable
8	Transparent	7.700±0.035	Stable
9	Transparent	7.913±0.162	Stable
10	Transparent	7.957±0.204	Stable
11	Transparent	9.087±0.065	Stable
12	Transparent	8.470±0.101	Stable
13	Transparent	7.893±0.097	Stable
14	Transparent	9.260±0.480	Stable

[a]Data are expressed as mean±SD (n = 3)

Thermodynamic stability was assessed through a series of tests, including a heating-cooling cycle, a centrifugation test, and a freeze-thaw cycle. During the heating-cooling cycle, we monitored the trends in pH and transmittance for each cycle, as illustrated in fig. 5.



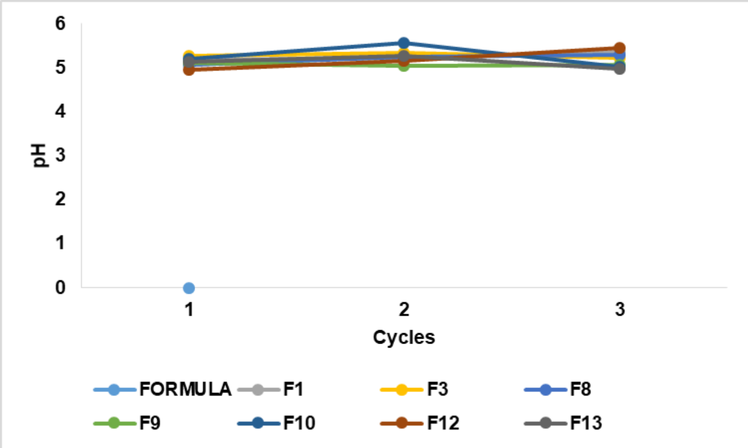
(A)



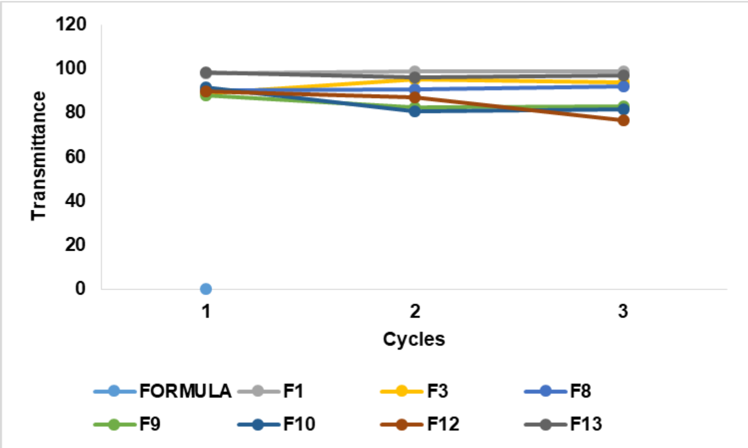
(B)

Fig. 5: (A) The trend of pH levels, (B) The trend of transmittance observed during the heating-cooling cycle

As illustrated in the fig. above, the pH values recorded during each cycle ranged from 4.02 to 5.36. Although these values are slightly outside the optimal pH range for skin, they remain within an acceptable threshold. All formulations demonstrated stability, exhibiting no separation during or after the heating-cooling cycle process. However, the transmittance of the nanoemulsion displayed notable variations, with formulations F2, F4, and F7 experiencing a decrease in transmittance and a transition to a milky white appearance, likely attributable to exposure to extreme temperatures. Furthermore, these specific formulations contained a higher oil phase compared to others, which may have contributed to the observed reduction in clarity, as evidenced by the color changes and decline in transmittance. From the initial tests, we selected seven formulations with transmittance values exceeding 95% for centrifugation testing, all of which exhibited no signs of separation. Subsequently, we proceeded with the final freeze-thaw test, as depicted in fig. 6.



(A)



(B)

Fig. 6: (A) The trend of pH levels, (B) the trend of transmittance observed during the freeze-thaw cycle

The data presented in the fig. above indicate that the pH of the nanoemulsion falls within the optimal range for skin pH. However, nanoemulsions F3, F8, F9, and F10 exhibited a reduction in transmittance to below 95%, suggesting an increase in droplet size as a consequence of exposuref extreme storage temperatures.

DISCUSSION

Essential oils are widely recognized for their antibacterial and antioxidant properties, underscoring the necessity for the development of formulations that incorporate these oils. The refractive index serves as an important parameter for assessing the purity and quality of an essential oil by comparing the measured value against established standards [22]. Typically, the refractive index of essential oils falls within the range of 1.450 to 1.590. Specifically, the essential oil derived from *M. alba* exhibits a refractive index of 1.449 at room temperature, which is marginally below the general range yet remains within acceptable limits. Furthermore, the solubility of essential oils in ethanol is another critical quality indicator; it is often observed to decrease in oils that have been improperly stored for prolonged periods [3].

The antibacterial efficacy of essential oils is influenced by their chemical composition [23]. According to gas chromatography-mass spectrometry (GC-MS) analysis, the primary constituents of *M. alba* essential oils include cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester, benzyl alcohol, linalool, and phenylethyl alcohol, which is consistent with findings from prior studies [3]. Linalool, a key compound, is widely distributed throughout the plant, particularly in the flowers, with concentrations ranging from 1.63% to 4.89% [24]. Comparable components are present in coriander essential oil (CEO), which contains linalyl acetate, geranyl acetate, and citronellal, with linalool being the predominant compound. Linalool demonstrates antibacterial properties by compromising the structural integrity of both Gram-positive and Gram-negative bacterial membranes, thereby increasing their permeability and resulting in the leakage of cellular contents [25]. The combination of CEO and gentamicin has been shown to act synergistically, enhancing the susceptibility of *Staphylococcus aureus* [26]. Benzyl alcohol exerts its antibacterial effects against Gram-positive bacteria by disrupting bacterial membranes, particularly at pH levels below 5. This disruption occurs through interference with the efflux pump, which increases membrane fluidity and diminishes the structural integrity of the bacterial membrane [27].

M. alba essential oil is composed of various constituents, including pinene and citronellol, which are also prevalent in other essential oils such as lemon, coriander, celery seed, rosemary, sage, thyme, citronella, and marjoram. These components have been shown to disrupt the lipid bilayer of bacterial cell membranes, resulting in the leakage of intracellular contents and ultimately leading to cell lysis, particularly in Gram-positive bacteria [28]. Investigations into other species within the Magnolia genus, specifically *M. sirindhorniae*, reveal significant inhibitory effects of its flower oil against strains of *Staphylococcus aureus* that are resistant to conventional antibiotics. Notably, the efficacy of *M. sirindhorniae* flower oil exceeds that of gentamicin and penicillin, both of which demonstrate no inhibitory effect against resistant strains of *S. aureus* [29].

The antibacterial properties of essential oils are primarily ascribed to their capacity to disrupt bacterial intracellular structures by increasing membrane permeability, which facilitates the penetration of oil components into bacterial cells. These components engage with hydrophobic sites of intracellular compounds, resulting in significant modifications to bacterial physiology. The formulation of nanoemulsions can enhance the delivery of these oils, as the reduced droplet size increases the surface area of bioactive compounds in contact with the cytoplasmic membrane, thereby promoting intracellular interactions and mitigating the risk of resistance development [30]. Based on the minimum inhibitory concentration (MIC) of *M. alba* essential oils, the composition of the *M. alba* nanoemulsion was optimized to include 2% champaca oil, 1% virgin coconut oil (VCO), 18% Tween 80, 9% polyethylene glycol (PEG) 400, and distilled water to achieve a total volume of 100 ml. VCO was selected as the oil phase due to its capacity to form a nanoemulsion system that incorporates essential oils [12]. Essential oils comprise a variety of phytochemicals, including terpenes (both mono-and sesquiterpenes), oxygenated derivatives (such as hydroxyl and carbonyl compounds), aliphatic aldehydes, alcohols, and esters [3]. In contrast, VCO is predominantly composed of fatty acids, particularly lauric acid [22]. Lauric acid functions as an emulsifying agent, reducing the interfacial tension of essential oils and facilitating their effective dispersion in an aqueous medium [31].

This study employs a combination of surfactants, specifically Tween 80 as the primary surfactant and PEG 400 as the co-surfactant. Non-ionic surfactants, such as these, exhibit minimal sensitivity to variations in pH and ionic strength, making them widely utilized in transdermal drug delivery due to their low toxicity and compatibility with a diverse range of ingredients [32]. The concentration of surfactants is critical in the formation of droplets during the preparation of nanoemulsions, as it influences the reduction of interfacial tension, the increase of interfacial area, and the decrease of the system's free energy. These factors collectively contribute to the formation of a nanoemulsion characterized by nanometer-sized droplets and enhanced thermodynamic stability [33]. Tween 80, possessing a higher hydrophilic character, effectively reduces interfacial tension and stabilizes the emulsion by creating a micelle-like structure around the oil droplets. In contrast, PEG 400 enhances the packing at the interface and further diminishes surface energy, thereby preventing droplet coalescence. The concentration of co-surfactants shouldn't exceed that of the surfactants, as co-surfactants can increase the solubility of water-insoluble drugs, which may subsequently lead to an increase in the droplet size of the emulsion preparation [34]. In this study, the ratio of surfactants to co-surfactants was maintained at 2:1, resulting in a hydrophilic-lipophilic balance (HLB) of 14.37. This specific HLB value has been demonstrated to facilitate a stable nanoemulsion preparation with droplet sizes less than 100 nm. The selected HLB values were aligned with the requirements of the oil phase, as higher HLB values are generally more suitable for oil-in-water nanoemulsions [9, 12].

The analysis conducted using Design Expert indicated that the components of oil, surfactant mixture (smix), and water adversely affect the transmittance response, with the surfactant mixture demonstrating a more pronounced impact. The combination of Tween 80 as the surfactant and PEG 400 as the co-surfactant, utilized in a 2:1 ratio, synergistically diminishes the interfacial tension between water and oil, thereby facilitating the formation of nanoemulsions characterized by nanometer-sized droplets. Smaller droplet sizes are indicative of a clear nanoemulsion, which can be both visually observed and quantitatively assessed through transmittance values approaching 100% [10,11]. Furthermore, Tween 80 and PEG 400 play a crucial role in preventing the coalescence of oil droplets, thereby inhibiting aggregation [35]. However, the application of excessively high concentrations of Tween 80 and PEG 400 may negatively impact transmittance values, leading to the formation of micelles in the continuous phase. This phenomenon increases osmotic pressure, promotes droplet aggregation, and accelerates the rate of Ostwald ripening [35–37]. Additionally, the oil component has a detrimental effect on the transmittance response; the incorporation of oil results in a more turbid preparation, which tends to exhibit a milky white appearance, as illustrated in formulations F2, F4, and F7.

The pH response analysis indicates that all components and their interactions exert a positive influence on the pH levels. Tween 80 and PEG 400, both of which contain hydroxyl groups, have the potential to elevate the pH of the formulation [31]. However, an increase in the concentrations of all components results in a reduction of pH. Virgin Coconut Oil (VCO), as one of the oil phases, contains acidic fatty acids, which tend to decrease the pH [22, 38]. While the combination of Tween 80 and PEG 400 positively affects the pH, an increase in their concentrations may lead to an adverse effect on the pH of the nanoemulsion [19].

Based on the analysis conducted using Design Expert software, the optimal formulation for the nanoemulsion was determined to consist of 4% oil phase, 27% surfactant mix (Smix), and 69% water phase. This formulation resulted in a pH of 5.33, a transmittance of 98.69%, a droplet size of 30.5 nm, a zeta potential of 1.21 mV, and a polydispersity index (PDI) of 0.026. These findings are consistent with the established criteria for topical nanoemulsions. It is important to note that the utilization of a single surfactant is inadequate for sufficiently reducing the interfacial tension between the aqueous and oil phases to form a stable nanoemulsion system. Consequently, the incorporation of short-chain amphiphilic molecules or co-surfactants is essential to achieve a reduction in surface tension to zero [21]. Conversely, excessive surfactant concentration may lead to the formation of mixed liquid crystals comprising surfactant, oil, and water, which complicates dispersion and results in larger droplet sizes [39]. The PDI value indicates the uniformity of droplet sizes, which can be attributed to the small droplet dimensions. This homogeneity is likely a result of the synergistic effects of Tween 80 and PEG 400 in reducing interfacial tension, thereby enhancing stability by minimizing the risk of aggregation [35, 40]. Additionally, the stirring process contributes to the fragmentation of larger oil droplets into smaller ones, while sonication is critical for achieving the desired final droplet size and uniformity, ensuring a monodisperse distribution [18]. The surface charge of the nanoemulsion is quantified by the zeta potential. The observed negative zeta potential is a result of the combination of Tween 80 and PEG 400. A low zeta potential suggests reduced colloidal stability, as stable nanoemulsions typically require a zeta potential exceeding ±30 mV. However, non-ionic surfactants such as Tween 80 and PEG 400 can provide stabilization through steric mechanisms, which prevent particle aggregation despite the low zeta potential. Unlike ionic surfactants, non-ionic surfactants do not generate electrostatic repulsion; instead, they form a protective layer around the droplets, thereby preventing coalescence and enhancing stability through steric hindrance [41]. This research demonstrated a low zeta potential; however, the thermodynamic stability assessments indicated no phase separation across all formulations, suggesting physical stability. Previous studies have similarly reported low zeta potential values while still demonstrating stable nanoparticle formulations [42, 43]. Therefore, the interpretation and application of zeta potential as a sole predictor of colloidal stability should be approached with caution, considering the role of surfactants and the various mechanisms influencing charge and interactions among them [34, 44].

The physical evaluation of nanoemulsions includes an assessment of organoleptic properties and viscosity. Viscosity is a critical parameter in topical formulations, as elevated viscosity can complicate application to the skin, lead to residue formation, and impede the release of active ingredients. The formulation's components—namely oil, Smix, and water—significantly influence the viscosity of the nanoemulsion. An increase in the proportion of water incorporated into the formulation correlates with a reduction in viscosity [12]. Formulations F2, F5, F11, and F14, which contain 69% water, exhibited a thicker consistency, as evidenced by their higher viscosity compared to other formulations.

This stability test was conducted to evaluate the physical quality of various formulations, specifically focusing on phase separation, pH, and transmittance under extreme storage temperatures and centrifugal forces. The cycles employed in this study were designed to simulate environmental conditions encountered during storage and handling, particularly in Indonesia, which experiences a tropical climate characterized by significant temperature fluctuations between day and night, as well as variations between air-conditioned and heated storage conditions. Such temperature fluctuations can induce physical changes in the formulation and alter interfacial tension, potentially leading to instability manifested as phase separation, creaming, or coalescence [45]. The findings of this study indicated that no visible phase separation occurred during or after the heating-cooling cycles. However, formulations F2, F4, and F7 exhibited a color change to milky white, suggesting possible physical instability in these nanoemulsions. These formulations also demonstrated a significant reduction in transmittance, which can be attributed to their high oil composition and limited surfactant combinations. The insufficient concentration of surfactants impeded the effective emulsification of the oil phase, as evidenced by the observed color change in the nanoemulsion and the decrease in transmittance. Furthermore, the increased lipophilicity associated with longer carbon chain lengths necessitates greater energy input or a higher surfactant mixture (smix) concentration to effectively reduce interfacial tension and achieve a stable nanoemulsion [33]. The pH of the formulations tended to be acidic, a result of the fatty acid content in virgin coconut oil (VCO) [38], with a notable decrease in pH observed during the heating-cooling tests.

The centrifugation test can enhance the migration rate of globules to the interfacial layer, promoting globule aggregation and potentially damaging the surfactant monolayer, which may lead to coagulation. Based on the results from the centrifugation tests, seven formulations did not exhibit phase separation, indicating their stability. In comparison to conventional emulsions, nanoemulsions require a larger quantity of surfactant. Furthermore, co-surfactants' involvement can optimize surfactants' effectiveness in preventing globule aggregation [46]. The combination of both surfactants and co-surfactants can create a nanoemulsion system with nanometer-sized droplets that are thermodynamically stable. The small droplet size can reduce the effects of gravitational forces and Brownian motion during centrifugation, thereby preventing creaming and sedimentation [47].

This study highlights the innovative use of *M. alba* oil with proven antibacterial activities against *S. aureus*. It could give information about the optimum formula of nanoemulsion that met the requirements of nanoemulsion and is thermodynamically stable. This study conducted limited optimization by varying the oil, smix, and water phases in ratios based on preliminary studies and previous research. The optimization results for pH and transmittance responses indicated that neither model produced significant data. This finding suggests that variations in these three components did not substantially affect the pH and transmittance responses before conducting thermodynamic stability tests. Both pH and transmittance exhibited drastic changes after the formulations were subjected to extreme temperatures over several cycles, and these parameters were not considered in the optimization process. Additionally, the research did not assess the changes in the characteristics of the nanoemulsion, including particle size, zeta potential, and polydispersity index during and after thermodynamic stability testing, thus preventing conclusions about the influence of stability on the characteristics of the nanoemulsion. Furthermore, this study established the ratio of surfactants to co-surfactants before optimization using HLB approaches and previous research. The ratio of surfactants to co-surfactants is a critical parameter for forming nanoemulsions, and it may influence both pH and transmittance values. The formulations achieved a clear nanoemulsion with transmittance greater than 95% and droplet sizes less than 100 nm with precise ratios.

CONCLUSION

M. alba essential oil exhibits significant antibacterial properties against Staphylococcus aureus, with a minimum inhibitory concentration (MIC) of 2%. These findings underscore its potential as a promising candidate for the formulation of an antibacterial topical nanoemulsion. The optimal composition of the *M. alba* nanoemulsion comprises 3.54% oil phase, 26.95% surfactant mix (Smix), and 69.51% aqueous phase. The formulation demonstrated a thermodynamically stable nanoemulsion with a pH of 5.513, a transmittance of 99.397%, a droplet size of 24.1 nm, a zeta potential of-1.14 mV, and a polydispersity index of 0.107. These results highlight the potential of *M. alba* essential oil-based nanoemulsions as viable candidates for the development of effective antibacterial topical formulations.

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AUTHORS CONTRIBUTIONS

All the authors made a substantial contributions to the research and the article. Conceptualization: [NNYM]; Methodology: [NNYM and MMVS]; Data Analysis: [MMVS]; Writing – Original Draft: [NNYM]; Writing – Review and Editing: [NNYM, IGMS, IGAAKW]; Supervision: [IGMS].

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest related to the material presented in this paper.

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