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Nanoemulsion stability of Javanese long pepper extract (Piper retrofractum Vahl): Effects of emulsion system and storage temperature

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Abstract. The objective of this study is to evaluate the stability of Javanese long pepper extract nanoemulsions at different temperatures and with varying Virgin Coconut Oil (VCO) to Tween 80 ratios. The study was conducted by using the variation of VCO with Tween 80 (1:9; 1:18; and 1:27), then stored at 4°C, 25°C and 40°C and then stored for 6 weeks. Nanoemulsions were observed for stability every week using % transmittance, pH and organoleptic parameters. The results of the % transmittance test and pH tests showed that the nanoemulsion remained stable at 4°C, while other temperatures showed less stability. The organoleptic test showed that there was no change in the treatment involving the concentration of Tween 80 : VCO (1:9) at a temperature of 4ºC (A1T1) and the concentration of Tween 80 : VCO (1:27) at a temperature of 4ºC (A3T1). The optimal treatment for nanoemulsion involves using a VCO to Tween 80 ratio of 1:18. This treatment results in a nanoemulsion size of 145.80 nm, a polydispersity index value of 0.7964, and a zeta potential value of -3.164 mV.

1 Introduction

Javanese long pepperis utilized as a primary ingredient in traditional medicine and cosmetics. The Javanese long pepper plant is composed of several compounds, including piperine, chavicin, palmatic acid, tetrahydropiperic acid, 1-undecylenyl-3, 4-methylledioxy benzene, piperidine, essential oil, N-isobutyldeka-trans-4-dienamide, and sesamin [1]. Pharmaceutical effects include analgesic (pain relievers), aphrodisiac (increased desire), diaphoretic (sweat remover), carminative (dispeller), sedative (tranquilizer), hematinic, and antelminic (worm medication) [2].

Piperine ($C_{17}H_{19}NO_3$) is an alkaloid in the form of a monocyclic prism with a melting point ranging from 128 to 130 °C. The active compound (piperine) extracted from Piper species in its application has the disadvantage of low solubility [3], [4] so that bioavailability is also low [3]. Low bioavailability causes the benefits of piperine in the body to be slow to be felt. Piperine exhibits high solubility in ethanol, while its solubility in water is comparatively low [5]. Insoluble compounds frequently exhibit partial absorption in water. Medicinal medicines with low water solubility exhibit sluggish dissolving rates, leading to reduced bioavailability (the proportion of pharmaceuticals that enter the circulatory system) [6]. Therefore, measures must be taken to enhance bioavailability. The efficacy of active compounds in Javanese long pepper is diminished due to their low solubility; therefore, nanoemulsion technology serves as a viable solution to this issue.

Nanoemulsions are employed as a means to enhance bioavailability. Nanoemulsions are thought to enhance the bioavailability of active substances under their diminutive size, hence promoting greater absorption of these compounds in the tiny intestinal wall [7]. The human body exhibits nearly 100% absorption of nanoherbal substances, but the absorption rate for micron-sized particles is about 5%. The nanoparticles' diminutive size facilitates their rapid dissolution and effective absorption in the intestine [7]. Nanoemulsions are preparations composed of an oil phase and an aqueous phase that are actually immiscible with a droplet size of about 100 nm, and they have a wide range of applications in drug delivery, food, cosmetics, pharmaceuticals, and material synthesis [8] The utilization of nanoemulsion technology has been widely developed for various purposes including simvastatin nanoemulsion for wound medicine [9], nanoemulsion containing active ingredients as anti-aging [10], nanoemulsion that can increase the bioavailability of curcumin [11], [12], [13], nanoemulsion increases the bioavailability of nutraceuticals, vitamins, and drugs [14], nanoemulsion that can increase antioxidant and antimicrobial activity [15]. Nano technology in Javanese long pepper extract has also been studied [16].

Nanoemulsions are produced within a drug delivery device called a Self-Nanoemulsifying Drug Delivery

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System (SNEDDS). SNEDDS combines oil, surfactant, cosurfactant, and active ingredient. When combined with water, it will create a nanoemulsion of the water-oil type [17]. Surfactants diminish the interfacial tension between two substances that do not mix well, facilitating the uniform dispersion of the internal phase and preventing the recombination of the dispersed internal phase [18]. Nanoemulsions have several benefits, including enhanced bioavailability of active components, regulated release of active substances, and improved sensory characteristics. The nanoemulsion with a size range of 50-200 nm exhibits enhanced absorption by the small intestinal wall, augmenting its bioavailability [7]. The properties of nanoemulsions are intricately linked to their physical stability and clarity, as these factors directly impact the size of the particles formed.

Nanoemulsions are produced by employing Tween 80 as an emulsifier and VCO as the oil component. Tween 80 offers the benefits of solubility in both water and ethanol. Additionally, it serves as a penetrating binder. Virgin Coconut Oil (VCO) is derived from the fresh fruit of the coconut tree. VCO is manufactured by a chemical-free and low-heat technique. VCO comprises several medium-chain fatty acids. Lauric acid is VCO's predominant medium-chain fatty acid. Prior studies investigated nanoemulsions containing different substances, including mangosteen fruit extract [17], temulawak [19], and moringa [7]. According to previous research, it is evident that the medicine requires an extended period during the preservation procedure, as it is not eaten instantly. It is crucial to consider the stability of nanoemulsions to choose the optimal formula for the storage process.

The stability of nanoemulsions is very important in their commercial use. The stability of nanoemulsions is seen from the physicochemical stability affected by unfavorable environmental conditions (including temperature, mechanical forces, and ionic forces) during production, storage, transportation and application [20]. The stability of nanoemulsions is important to maintain so that the solution remains nano-sized, which has implications for increasing the bioavailability of piperine content, so that the effects of piperine are quickly felt by the body. Despite the known benefits of nanoemulsions, there is limited research on the stability of Javanese long pepper extract nanoemulsions under varying storage conditions. This study aims to evaluate the stability of nanoemulsions of Javanese long pepper extract at different temperatures and with various ratios of VCO and Tween 80, which is crucial for their effectiveness in pharmaceutical and cosmetic applications. The stability of nanoemulsions has a very important contribution to the food, pharmaceutical and cosmetic industries as well as the public in increasing the biovaibility of piperine compounds in Javanese long pepper.

2 Method

2.1 Materials and tools

The primary components utilized in this study consist of Javanese long pepperes that were collected after they reached 90 days of maturity and dried using a cabinet drier set at 40°C [21]. Other materials include 96% ethanol, filter paper, Tween 80 (MERCK 822187), virgin coconut oil (VCO), and distilled water. The study utilized various tools, including scales, measuring cups, vial bottles, funnels, spatulas, drop and volume pipettes, porcelain cups, Erlenmeyer flasks, glass beakers, a hot plate (Thermo Scientific Sp88851705 Cimarec), a magnetic stirrer, a UV vis spectrophotometer (Genesys 10-s USA), a pH meter, and a rotary evaporator.

2.2 Research design

The study employed a Randomized Group Design with 2 components. Factor 1 was the ratio of VCO to Tween 80, which had 3 values (1:9, 1:18, 1:27). The storage temperature factor, denoted as Factor 2, has three levels: 4°C, 25°C, and 40°C, as reported by Jusnita et al. [19]. These levels are presented in Table 1. The experiment was replicated twice for each treatment.

Table 1. Research design.

Ratio VCO : Tween 80 (g)	Storage temperature		
	4°C (T1)	25°C (T2)	40°C (T3)
1 : 9 (A1)	A1T1	A1T2	A1T3
1 : 18 (A2)	A2T1	A2T2	A2T3
1 : 27 (A3)	A3T1	A3T2	A3T3

2.3 Javanese long pepper extract

The extraction procedure of Javanese long pepper involved the following approach described by Rahmanet al. [22]. Specifically, 200 g of Javanese long pepper was placed in the chamber of the Pulsed Electric Field (PEF) device. The extraction procedure involves using PEF pretreatment at a voltage of 5000 volts and a frequency of 3 kHz for 480 seconds. Additionally, Javanese long pepperwas supplemented with a 96% ethanol solvent at a 1:3 ratio. After being macerated for 24 hours, the initial 6 hours involve stirring or shaking. First, filtration is performed to obtain filtrate I. Then, the remaining residue is dissolved once more using leftover ethanol solvent I and subjected to maceration. Subsequently, the mixture is filtered to yield filtrate II. Filtrate I and II are then combined and subjected to evaporation using an evaporator set at a temperature of 50°C.

2.4 Nanoemulsion synthesis of Javanese long pepper extract

The nanoemulsion synthesis of Javanese long pepper extract, as described by Ratnapuri et al. [23], involves the combination of virgin coconut oil (VCO), Javanese long pepper extract, and Tween 80 utilizing a magnetic stirrer at 1000 rpm for 10 minutes. The VCO, Javanese long pepper extract, and Tween 80 were combined in the proportions specified in Table 1. Subsequently, distilled water was incrementally introduced until the total volume reached 100 ml. The procedure involves adding distilled water while stirring with a magnetic stirrer set at 1250 revolutions per minute for 10 minutes.

2.5 Stability of Javanese long pepper extract nanoemulsion at different storage conditions

The stability of the nanoemulsion was assessed by storing it under present circumstances. The storage process was conducted at three distinct temperatures: 4ºC, 25ºC, and 40ºC. The nanoemulsion was kept at a low temperature of 4ºC and placed in the refrigerator. The second temperature was set to the standard room temperature of 25ºC, while the last was set to 40ºC and maintained in an incubator. Data was collected weekly for 6 weeks.

2.6 Test percent transmittance of Javanese long pepper extract nanoemulsion

The percent transmittance of a nanoemulsion of Javanese long pepper extract was determined using the method described by Zulfa et al. [24]. A 1 mL quantity of Javanese long pepper extract was dissolved in a 100 mL volumetric flask using distilled water to create a nanoemulsion. The % transmittance of the solution was determined by measuring it using a spectrophotometer at a wavelength of 650 nm. Aquadest served as a blank solution.

2.7 pH test of Javanese long pepper extract nanoemulsion

The pH testing was conducted utilizing the methodology described by Shoviantari et al. [25]. The pH of nanoemulsion formulations of Javanese long pepper extract was determined using a digital pH meter. Before evaluating the preparation's pH, the pH meter was calibrated using pH 4 and 7 buffer solutions to guarantee its correct functioning. Upon completing the calibration process, the electrode was thoroughly cleansed and subsequently placed into a glass beaker containing the nanoemulsion of Javanese long pepper extract. The pH value of the nanoemulsion formulation of Javanese long pepper extract will be displayed on the pH meter screen.

2.8 Organoleptic test of Javanese long pepper extract nanoemulsion

An organoleptic evaluation was conducted on a nanoemulsion of Javanese long pepper extract using the approach described by Shoviantari et al. [25]. Conduct an organoleptic test by preparing a nanoemulsion solution. Conduct visual examinations of color, scent, and sediment alterations once weekly for six weeks.

2.9 Particle size, polydispersity index, and zeta potential test

The scientific publication by Budiarto et al. [26] was altered by doing particle size testing utilizing a Particle Size Analyzer (PSA). A volume of 1 mL of the nanoemulsion preparation was transferred into a cuvette. Subsequently, the particle size, polydispersity index, and Zeta potential were determined using a Particle Size Analyzer (PSA) known as the Malvern Zetasizer Nano ZS.

2.10 Data analysis

The data was analyzed using ANOVA (Analysis of Variance) at a 95% confidence level or a significance threshold of 0.05 to assess the impact of each treatment by SPSS version 25. Additional Duncan Multiple Range Test (DMRT) tests were conducted to determine if each element had a statistically significant impact.

3 Result and discussion

3.1 Stability of Javanese long pepper extract nanoemulsion based on transmittance value

The nanoemulsion of Javanese long pepper extract's stability during six weeks of storage can be observed in detail in Figure 1, which is based on the transmittance value. The stability of the nanemulsion based on percent transmittance showed stable clarity. As the purpose of nanoemulsion formation is expected to have high clarity, good physical stability against gravitational separation and droplet aggregation, and increased bioavailability of substances. According to Montes de Oca-Ávalos et al [27], high transparency is a very favorable and attractive characteristic of nanoemulsions because the small droplet size ensures weak light scattering in the final product.

The percent transmittance value produced in this study ranged from 86.3% to 87.9%. Some previous studies of artemether nanoemulsion had a percent transmittance of 98.20 ± 0.7% [28], amphotericin B nanoemulsion with a percent transmittance of >97% [29], nanoemulsion containing c-phenylcyclic-[4]-resorcinyl octasinnamate and c-methyl-cyclic-[4]-resorcinyl octabenzoate had a percent transmittance of 90.84% [30].

Figure 1a displays the variation in the percentage of light transmitted through a nanoemulsion created with a VCO:Tween 80 ratio of 1:9, stored at temperatures of 4ºC, 25ºC, and 40ºC. Storage at a temperature of 4ºC exhibited variations in transmittance value between week 1 and week 4. However, there was a decrease in transmittance value between week 4 and week 6 (Figure 1a). The rise in the transmittance percentage is attributed to alterations in the clarity's shape due to the particles in the preparation reassembling to form bigger globules at lower temperatures [31]. The nanoemulsion of Javanese long pepper extract maintained at 25ºC exhibited an increasing trend in percent transmittance value from week 1 to week 6. Employing a temperature of 25ºC resulted in an elevation of the percent transmittance value, while no significant statistical difference was seen. According to Redhita et al., [32], the percentage of light transmitted increases under hot climatic circumstances.

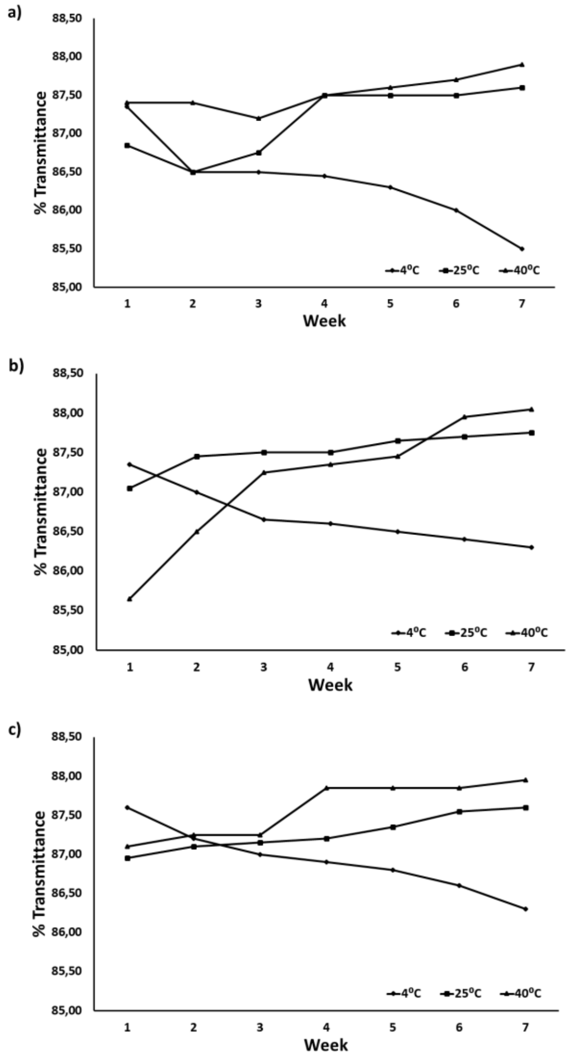

Fig 1. Percent Transmittance values during storage at different temperatures; a) nanoemulsion prepared with VCO:Tween 80 ratio (1:9); b) nanoemulsion prepared with VCO:Tween 80 ratio (1:18); and c) nanoemulsion prepared with VCO:Tween 80 ratio (1:27).

Moreover, Indalifiany et al. [33] elucidated a direct correlation between the percent transmittance value and the size of the nanoemulsion droplets, whereby a greater percent transmittance value indicates a smaller droplet size. The high transmittance percentage of the nanoemulsion indicates that it appears clear due to its minimal droplet size, allowing the light beam to pass through easily and resulting in a high transmittance reading. At a temperature of 40ºC, the percent transmittance value declined from week 1 to week 2 but increased from week 3 to week 6. This phenomenon occurs because the use of elevated temperatures leads to an augmentation in the amount of clarity. Redhita et al., [32], found that the percentage of light transmission increases at high temperatures. Moreover, Adi et al. [34] attributed the observed rise to alterations in the dimensions of the globules, which expanded within the nanoemulsion.

The nanoemulsion of Javanese long pepper extract, prepared with a VCO:Tween 80 ratio of 1:18, exhibited variations in the percent transmittance value when stored at different temperatures (Figure 1b). The nanoemulsion maintained at a temperature of 4ºC exhibited an increase in transmittance value between week 1 and week 2, followed by a subsequent drop between week 2 and week 6. It was a result of utilizing low temperatures. Increased temperatures in the environment will result in more significant values of % transmittance, as stated by Redhita et al., [32]. The reduction in transmittance percentage at 4ºC is attributed to the enlargement of the droplet size in the nanoemulsion, which hinders the passage of the light beam [31]. The Javanese long pepper extract nanoemulsion maintained at 25ºC was inclined to elevate the percent transmittance value with time. Utilizing room temperature increased the % transmittance value, but the difference was insignificant. The Javanese long pepper extract nanoemulsion maintained at 40ºC exhibited an inclination to elevate the percent transmittance value with time. This phenomenon occurs due to the utilization of elevated temperatures, which leads to an augmentation in the level of clarity. This phenomenon aligns with the findings of Redhita et al., [32], which indicate that the ambient temperature strongly influences the transparency of nanoemulsion. The high percent transmittance results of nanoemulsions indicate that nanoemulsions at 25ºC and 40ºC exhibit clarity due to their minimal droplet size [33].

The percentage change in transmittance value of the nanoemulsion, which was prepared using a VCO:Tween 80 ratio of 1:27 throughout storage, is illustrated in Figure 1c. The transmittance value of the nanoemulsion decreased when kept at 4ºC. This phenomenon arises due to the use of low temperatures, which induces alterations in the transparency of the nanoemulsion. As a result, the particles in the nanoemulsion aggregate to create larger globules [31]. At a temperature of 25ºC, the nanoemulsion exhibited an upward trend in the percent transmittance value throughout storage. Utilizing room temperature resulted in a higher percent transmittance value, but this gain was not statistically significant. The percent transmittance value of the nanoemulsion held at 40ºC exhibited an increasing trend over time. This phenomenon arises due to the external temperature stimulus [32], leading to the formation of minuscule droplets [33].

At higher temperatures, the viscosity of the liquid usually decreases, so the particles in the emulsion can move more freely and more easily combine into aggregates. Increased temperature can also cause surfactants to degrade or change structure, which can reduce the ability of surfactants to cover the surface of particles and prevent aggregation [35]. The particles in nanoemulsions often have a stable electrical charge due to the presence of surfactants or polyelectrolytes. Temperature can affect the electrostatic interactions between the particles. At higher temperatures,

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electrostatic interactions may decrease, making it easier for the particles to combine. In addition, temperature can also affect the kinetics of chemical reactions that occur in nanoemulsion systems. Chemical reactions that take place at high temperatures can increase the activity and mobility of particles, making aggregation easier to occur [35].

3.2 Stability of Javanese long pepper Extract Nanoemulsion Based on pH Value

The stability of the nanoemulsion of Javanese long pepper extract can be assessed by observing the pH value changes when stored under various temperature settings. Storage at different temperatures during storage did not have a statistically significant effect. This indicates that the nanoemulsion based on the pH parameter is stable. Figure 2a shows the nanoemulsion produced using a VCO:Tween 80 ratio of 1:9 at a temperature of 4ºC exhibited a pH range of 6.085 - 6.135. The stability of nanoemulsion is enhanced when stored at a temperature of 4ºC. This phenomenon aligns with the findings of Sun et al. [36], who reported that nanoemulsions exhibit stability even under specific temperature conditions. In addition, Jafari and McClement [37] stated that oil-in-water nanoemulsions with a pH range of 6-8, when formulated with surfactants, will acquire a negative charge. This charge inhibits the droplets from coming close to each other, ensuring the nanoemulsion's stability. The pH of the nanoemulsion held at 25ºC ranged from 6.070 to 6.020. The pH value consistently decreases weekly due to increasing temperature and a drop in the nanoemulsion value. The nanoemulsion held at a temperature of 40ºC ranges from 5.920 to 5.880. The elevated temperature employed had a significant impact on reducing the pH level of the nanoemulsion. The stability of nanoemulsions is greatly influenced by temperature, and as the temperature rises, the stability of the nanoemulsion diminishes [36]. This phenomenon arises due to the utilization of vegetable oil as the oil component, leading to the hydrolysis of fatty acid esters into breakdown products of free fatty acids. Furthermore, elevated temperatures induce hydrolysis, leading to a decline in the pH of the nanoemulsion [38].

The nanoemulsion of Javanese long pepper extract created with a VCO:Tween 80 ratio of 1:18 is illustrated in Figure 2b at various storage conditions. The pH of the nanoemulsion kept at 4ºC ranged from 6.380 to 6.465. The nanoemulsion exhibited a consistent and unchanging pH level. Sun et al. [36] stated that temperature treatment stabilizes the pH of nanoemulsions. The pH of nanoemulsion is below neutral or slightly acidic. The presence of acidity leads to a mild repulsion among molecules and droplets, resulting in the maintenance of steady pH levels even under severe temperatures. Jusnita et al. [19] elucidated that nanoemulsions with a pH range of 6-8, formulated with surfactants, exhibit a negative charge. This charge effectively hinders the droplets from coming into proximity to one another, hence ensuring the stability of the nanoemulsion. In addition, the pH of nanoemulsion kept at 25ºC ranges from 6.260 to 5.900. The pH of nanoemulsion tends to fall. The nanoemulsion held at a temperature of 40ºC exhibited pH values ranging from 6.085 to 5.630. The pH at 40ºC was lower than the prior storage conditions of 4ºC and 25ºC. This phenomenon happens due to the hydrolysis of fatty acid esters in the oil component, resulting in the formation of degradation products in the form of free fatty acids. Furthermore, elevated temperatures induce hydrolysis, leading to a decline in the pH of nanoemulsions [38].

The nanoemulsion of Javanese long pepper extract made with VCO:Tween 80 ratio (1:27) at different storage temperatures can be seen in Figure 2c. The pH of the nanoemulsion kept at 4ºC ranged from 6.280 to 6.335. There was no noticeable variation in this state over the 6-week storage period. The stability of nanoemulsion treatment is enhanced at a temperature of 4ºC. The pH of nanoemulsion kept at 25ºC ranges from 6.090 to 5.800. The pH value decreases when the storage temperature increases to 4ºC due to increasing temperature and a drop in the nanoemulsion value. The nanoemulsion held at a temperature 40ºC yields values ranging from 6.145 to 5.605. The pH measurements did not show statistically significant differences when stored at different temperatures for 6 weeks. It indicates that the pH remained stable during the storage period.

Citrus oil nanoemulsion at surfactant ratio and surfactant type significantly affected the formation and stability of nanoemulsion at a certain pH [39]. Nanoemulsion of *Woodfordia fruticosa* extract showed higher stability at varying pH ranges [40]. Nanoemulsion of hazelnut oil showed that changes in pH value and viscosity of the preparation can affect the stability of the nanoemulsion [41]. According to Jafari and McClement [37], the stability of nanoemulsion is maintained by the presence of surfactants, which create a negative charge that inhibits the droplets from coming close to each other, thereby ensuring the stability of the nanoemulsion.

The performance of surfactants, in stabilizing nanoemulsions is pH-dependent. Non-ionic surfactants, such as Tween, can undergo solubility changes with pH, which affects their ability to stabilize emulsions. Higher surfactant concentrations at a given pH level can increase stability [39]. Changes in pH can affect the droplet size and polydispersity index (PDI) of nanoemulsions. Previous research on nanoemulsions of Woodfordia fruticosa extract showed that different pH ranges affect the stability of nanoemulsions. Different pH levels affect the stability of droplet size and zeta potential value of nanoemulsions [40].

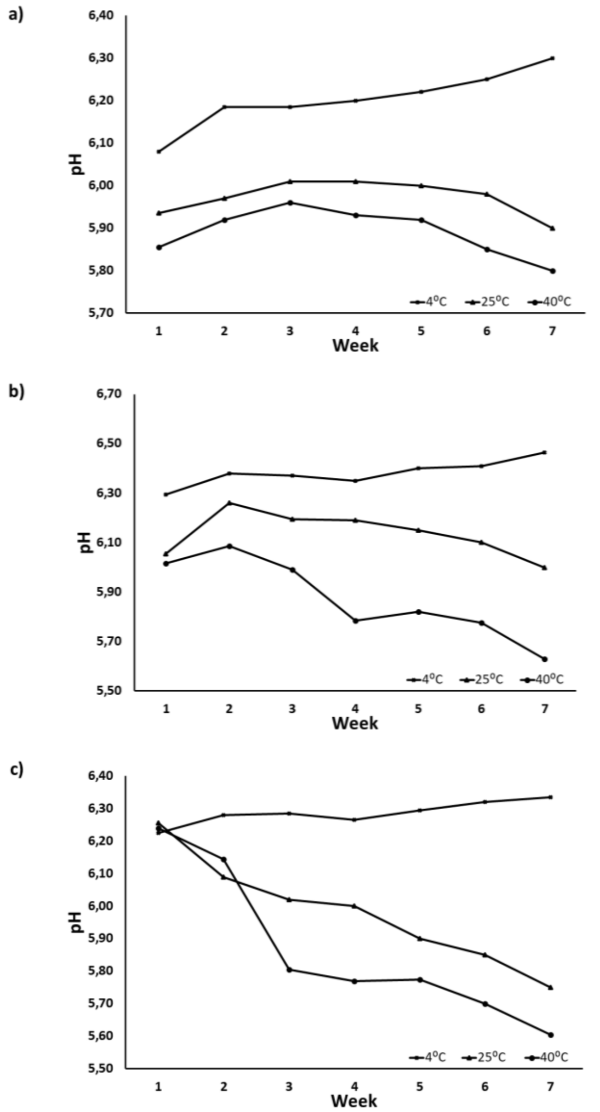

Fig 2. pH value during storage at different temperatures; a) nanoemulsion prepared with VCO:Tween 80 ratio (1:9); b) nanoemulsion prepared with VCO:Tween 80 ratio (1:18); and c) nanoemulsion prepared with VCO:Tween 80 ratio (1:27).

The pH affects the ionic strength of the solution, thus affecting the electrostatic interactions between droplets. Higher ionic strength can reduce electrostatic repulsion between droplets, leading to droplet coalescence and low stability. Conversely, maintaining a stable pH can help maintain electrostatic repulsion, leading to increased stability of the nanoemulsion [42].

3.3 Stability of Javanese long pepper extract nanoemulsion based on organoleptic value

An organoleptic test is a sensory evaluation method that utilizes the five senses to provide a detailed description of nanoemulsions. Organoleptic tests encompass the evaluation of the shape or consistency, color, and odor of the final preparation [25]. The intense, deep brown color and distinctive scent of Javanese long pepper extract influence the color and aroma of nanoemulsions. The objective of the organoleptic test is to assess any sensory changes in the nanoemulsion of Javanese long pepper extract after 6 weeks of storage at three different temperatures: 4ºC, 25ºC, and 40ºC.

Organoleptic observations indicated that storing the mixture of virgin coconut oil (VCO) and Tween 80 at different ratios at a temperature of 4ºC resulted in a consistent nanoemulsion. There were no noticeable changes in color, odor, or phase. This information can be found in Table 2, Table 3, and Table 4. A consistent color over time indicates a stable nanoemulsion preparation [43], [44]. Color consistency is of great concern especially in pharmaceutical and cosmetic preparations. The taste and odor of nanoemulsion should be acceptable and consistent. An unpleasant taste or odor may indicate instability or contamination [45], [46]. Previous research showed that nanoemulsion of nutmeg essential oil had a clear and stable appearance [44], nanoemulsion of citronella oil showed clear organoleptic characteristics and no phase separation [43].

Furthermore, this phenomenon aligns with the findings of Arianto & Cindy [47], who observed that a sunflower oil nanoemulsion remained stable throughout a 12-week storage period at three different temperatures: room temperature (25 ± 2°C), low temperature (4 ± 2°C), and high temperature (40 ± 2°C). Throughout the 12-week storage trial at different temperatures, the nanoemulsion did not alter color, consistency, odor, or phase separation. However, the emulsion had a color shift after 8 weeks of storage and showed phase separation (indicating instability) for the whole 12-week period at ambient temperature. Rismarika et al. [48] conducted a study that created a durable kepayang oil nanoemulsion, which maintained its odor, color, and clarity properties for 4 weeks. The research conducted by Redhita et al. [32] yielded a nanoemulsion of basil leaf extract that exhibited color, odor, and phase stability during a storage period of 4 days. In their study conducted in Widyastuti and Saryanti [49] developed a stable garlic extract nanoemulsion that exhibited no alterations in color, flavor, taste, clarity, and phase separation over the storage period. The organoleptic properties of nanoemulsions strongly influence consumer acceptability. Therefore, organoleptic consistency will be closely related to consumer acceptability.

Table 2. Observation of nanoemulsion during storage in terms of color.

Treatment	Week					
	1	2	3	4	5	6
A1T1	O	O	O	O	O	O
A1T2	O	O	O	Y	Y	Y
A1T3	O	O	O	O	O	O
A2T1	O	O	O	O	O	O
A2T2	O	O	O	O	O	O
A2T3	O	O	O	O	O	O
A3T1	O	O	O	O	O	O
A3T2	O	O	O	O	Y	Y
A3T3	O	O	O	O	O	O

Description: O: Orange, and Y: Yellow.

Table 3. Observation of nanoemulsion during storage in terms of odor.

Treatment	Week					
	1	2	3	4	5	6
A1T1	N	N	N	N	N	N
A1T2	A	A	A	A	A	A
A1T3	N	A	A	A	A	A
A2T1	N	N	N	N	N	N
A2T2	N	A	A	A	A	A
A2T3	N	A	A	A	A	A
A3T1	N	N	N	N	N	N
A3T2	A	A	A	A	A	A
A3T3	N	A	A	A	A	A

Description: N: Normal and A: Acid.

Table 4. Observation of nanoemulsion during storage in terms of precipitation.

Treatment	Week					
	1	2	3	4	5	6
A1T1	-	-	-	-	-	-
A1T2	+	+	+	+	+	+
A1T3	-	-	-	-	-	-
A2T1	+	+	+	+	+	+
A2T2	+	+	+	+	+	+
A2T3	+	+	+	+	+	+
A3T1	-	-	-	-	-	-
A3T2	+	+	+	+	+	+
A3T3	+	+	+	+	+	+

Description: - No precipitates, dan + There is precipitate.

Table 5. Nanoemulsion Characteristics at Different Ratios of Tween 80 : VCO.

Treatment	Size of particle (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
VCO: Tween 80 (1:9)	173,98	0,9078	-1,464
VCO: Tween 80 (1:18)	145,80	0,7964	-3,164
VCO: Tween 80 (1:27)	375,20	0,4532	-1,567

3.4 Particle Size, Polydispersity Index (PDI), and zeta potential

The nanoemulsion of Javanese long pepper extract, prepared with a VCO:Tween 80 ratio of 1:18, resulted in particles with a size of 145.8 nm, as indicated in Table 5. The superiority of the nanoemulsion over the other treatments can be attributed to its smaller size. Decreasing the size of the nanoparticles leads to improved performance. According to Aprilya et al. [50], medication formulations with reduced particle sizes have a greater chance of reaching specific cells due to the increased surface area of the droplets. In addition, Ardian et al. [51] said that an optimal nanoemulsion size falls between the particle size range of 50-500 nm. The particle size of nanoemulsion is closely related to the ability of nanoemulsion as a carrier of active compounds into the body. The smaller the size indicates the ability to be quickly absorbed in the body is greater. The particle size of a stable nanoemulsion usually ranges from 10 to 200 nm [43], [46], [52].

The nanoemulsion of Javanese long pepper extract, produced with a VCO:Tween 80 ratio of 1:27, exhibited a superior formula due to its low polydispersity index (PDI) value, which was near 0 (Table 5). Aini [53] states that a desirable nanoemulsion should have an almost zero polydispersity index value. Moreover, according to Indriani [54], a polydispersity index value close to zero signifies a uniform distribution of particles, whereas a number close to one implies a significant level of heterogeneity. Agglomeration occurs as a result of particle collisions caused by the presence of a heterogeneous particle distribution. Small particle size distribution (low PDI) shows better stability [43], [46], [52]. A low PDI value has implications for nanoemulsions that do not change easily, thus facilitating their application as drug carriers.

The zeta potential values of the nanoemulsion of Javanese long pepper extract, prepared with a VCO:Tween 80 ratio, varied from -3.164 mV to -1.464 mV (Table 5). Imanto et al. [55] reported that the zeta potential values range from +30 mV to -30 mV. A zeta potential of +30 mV signifies a significant degree of stability in the nanoemulsion. In contrast, a zeta potential of -30 mV is sufficient to preserve stability in the dispersion system of the nanoemulsion. This phenomenon arises due to its ability to inhibit the formation of particle agglomerates by exerting repulsive forces on the surface of the particles. Moreover, Imanto et al. [55] elucidated that the diminished zeta potential value resulted from the presence of Tween 80, which is an uncharged nonionic surfactant attached to the hydrophobic group. As a result, the surfactant causes the oil particles' surface to become neutral. Negative or positive zeta potential values can help stabilize nanoemulsions by preventing aggregation. A zeta potential value close to zero indicates poor stability [46], [52], will cause the nanoemulsion to undergo changes that will reduce the nanoemulsion functionally.

Previous research produced nanoemulsions of Nigella Sativa L. seed oil with a droplet size of 59. 2 nm [52], citronella oil nanoemulsion with droplet size 8.96 - 10.07 nm [43], erythromycin nanoemulsion with particle size 170.6 nm, polydispersity index (PDI) 0.403, and zeta potential -8.8667 mV [46], eugenol nanoemulsion with particle size 50.8 - 248 nm, polydispersity index (PDI) 0.242 - 1, and zeta potential -23.2 to -16.2 mV [56], clove essential oil nanoemulsion with particle size 29.1 nm, PDI 0.026, and zeta potential -31.4 mV [57].

4 Conclusion

The stability of the nanoemulsion was determined by analyzing the results of the transmittance test and pH test conducted over six weeks. It was found that the nanoemulsion remained stable while stored at a temperature of 4ºC. The organoleptic test showed no change in the nanoemulsion when the ratio of VCO to Tween 80 was 1:9 and stored at a temperature of 4ºC

(A1T1), as well as when the ratio was 1:27 and stored at the same temperature (A3T1). The most effective treatment for nanoemulsion was achieved using a VCO:Tween 80 ratio of 1:18. This resulted in a nanoemulsion size of 145.80 nm, a polydispersity index value of 0.7964, and a zeta potential value of -3.164 mV.

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References

1. W. Haryudin and O. Rostiana, *Stabilitas Karakter Morfologi 10 Aksesi Cabe Jawa (Piper retrofractum Vahl.) di Kebun Percobaan Cikampek*, Bul. Littro, **22**, 1 (2011)
2. R. Evizal, *Status Fitofarmaka Dan Perkembangan Agroteknologi Cabe Jawa (Piper Retrofractum Vahl.)*, Jurnal Agrotropika, **18**, 1 (2013)
3. D. Anissian, M. Ghasemi-Kasman, M. Khalili-Fomeshi, A. Akbari, M. Hashemian, S. Kazemi, and A. A. Moghadamnia, *Piperine-loaded chitosan-STPP nanoparticles reduce neuronal loss and astrocytes activation in chemical kindling model of epilepsy,* Int J Biol Macromol, **107**, (2018)
4. M. R. Bhalekar, A. R. Madgulkar, P. S. Desale, and G. Marium, *Formulation of piperine solid lipid nanoparticles (SLN) for treatment of rheumatoid arthritis*, Drug Dev Ind Pharm, **43**, 6 (2017)
5. N. P. E. Hikmawanti, E. Hanani, S. Maharani, and A. I. W. Putri, *Kadar Piperin Ekstrak Buah Cabe Jawa dan Lada Hitam dari Daerah dengan Ketinggian Berbeda*, Jurnal Jamu Indonesia, **6**, 1 (2021)
6. Z. D. Siqhny, M. N. Azkia, and B. Kunarto, *Karakteristik Nanoemulsi Ekstrak Buah Parijoto (Medinilla speciosa Blume)*, Jurnal Teknologi Pangan dan Hasil Pertanian, **15**, 1 (2020)
7. N. Jusnita and W. S. Tridharma, *Karakterisasi Nanoemulsi Ekstrak Daun Kelor (Moringa oleifera Lamk.)*, Jurnal Sains Farmasi & Klinis, **6**, 1 (2019)
8. A. Gupta, H. B. Eral, T. A. Hatton, and P. S. Doyle, *Nanoemulsions: Formation, properties and applications*, Soft Matter, **12**, 11 (2016)
9. H. Amoozegar et al., *A novel formulation of simvastatin nanoemulsion gel for infected wound therapy: In vitro and in vivo assessment,* J Drug Deliv Sci Technol, **72**, 69 (2022)
10. F. Xue, X. Li, L. Qin, X. Liu, C. Li, and B. Adhikari, *Anti-aging properties of phytoconstituents and phyto-nanoemulsions and their application in managing aging-related diseases*, Adv Drug Deliv Rev, **176**, (2021)
11. A. A. Esperón-Rojas, R. Baeza-Jiménez, D. Santos-Luna, L. del C. Velasco-Rodríguez, L. R. Ochoa-Rodríguez, and H. S. García, *Bioavailability of curcumin in nanoemulsions stabilized with mono- and diacylglycerols structured with conjugated linoleic acid and n-3 fatty acids*, Biocatal Agric Biotechnol, **26**, (2020)
12. R. Vecchione et al., *Curcumin bioavailability from oil in water nano-emulsions: In vitro and in vivo study on the dimensional, compositional and interactional dependence*, Journal of Controlled Release, **233**, (2016)
13. F. Garavand, M. Jalai-Jivan, E. Assadpour, and S. M. Jafari, *Encapsulation of phenolic compounds within nano/microemulsion systems: A review*, Food Chem, **364**, (2021)
14. R. Zhang, Z. Zhang, and D. J. McClements, *Nanoemulsions: An emerging platform for increasing the efficacy of nutraceuticals in foods*, Colloids Surf B Biointerfaces, **194**, (2020)
15. A. M. Pisoschi, A. Pop, C. Cimpeanu, V. Turcuş, G. Predoi, and F. Iordache, *Nanoencapsulation techniques for compounds and products with antioxidant and antimicrobial activity - A critical view,* Eur J Med Chem, **157**, (2018)
16. A. Rahman, S. H. Sumarlan, Sukardi, and D. Pranowo, *Optimization of Javanese Long Pepper (Piper retrofractum Vahl) extract nanoparticles as an aphrodisiac preparation*, in E3S Web of Conferences, EDP Sciences, Mar. (2024)
17. N. W. M. Lina, M. R. Sutharini, N. P. A. D. Wijayanti, and K. W. Astuti, *Karakteristik Nanoemulsi Ekstrak Kulit Buah Manggis (Garcinia mangostana L.)*, Jurnal Farmasi Udayana, **6**, 1 (2017)
18. A. S. Herbianto, *Pengaruh Perbedaan Konsentrasi Surfaktan Terhadap Karakter Fisik dan pH Nanoemulsi Pencerah Kulit, Calyptra,* Jurnal Ilmiah Mahasiswa Universitas Surabaya, **7**, 1 (2018)
19. N. Jusnita, W. Syurya, and M. S. Pepertua Diaz, *Formulasi Nanoemulsi Ekstrak Temulawak (Curcuma xanthorrhiza Roxb) dengan Metode Inversi Suhu*, Jurnal Farmasi Higea, **11**, 2 (2019)
20. Q. Liu, H. Huang, H. Chen, J. Lin, and Q. Wang, *Food-grade nanoemulsions: Preparation, stability and application in encapsulation of bioactive compounds*, Molecules, **24**, 23 (2019)
21. A. Rahman, S. H. Sumarlan, and D. Pranowo, *Quality of Javanese long pepper (Piper retrofractum Vahl) simplicia harvested at different maturity stages and drying temperatures*, Emir J Food Agric, **35**, 12 (2023)
22. A. Rahman, H. Fansuri, B. Diyah Probowati, A. Miftah, and M. Sa'diyah, *Efek perlakuan awal dengan pulsed electrik field (PEF) terhadap kualitas ekstrak cabe jamu (Piper retrofractum Vahl.)*, AGROINTEK: Jurnal Teknologi Industri Pertanian, **17**, 4 (2023)
23. P. H. Ratnapuri et al., *Formulasi dan Evaluasi Nanoemulsi dari Ekstrak Herba Kelakai dengan Kombinasi Tween 80 dan Propilenglikol*, Prosiding

Seminar Nasional Lingkungan Lahan Basah , **7**, 2 (2022)

24. E. Zulfa, D. Novianto, and D. Setiawan, *Formulasi Nanoemulsi Natrium Diklofenak dengan Variasi Kombinasi Tween 80 Dan Span 80 : Kajian Karakteristik Fisik Sediaan*, Media Farmasi Indonesia, **14**, 1 (2019)
25. F. Shoviantari, Z. Liziarmezilia, A. Bahing, and L. Agustina, *Uji Aktivitas Tonik Rambut Nanoemulsi Minyak Kemiri (Aleurites moluccana L.),* Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia, **6**, 2 (2020)
26. W. Budiarto, N. Nuur Rochmah, and L. Setiyabudi, Formulasi Sediaan Nanoemulsi Ekstrak Daun Mangrove Avicennia Marina dengan Virgin Coconut Oil sebagai Fase Minyak, *Jurnal Ilmiah Jophus : Journal of Pharmacy UMUS*, **2**, 01 (2020)
27. J. M. Montes de Oca-Ávalos, R. J. Candal, and M. L. Herrera, Nanoemulsions: stability and physical properties, *Current Opinion in Food Science*, **16** (2017)
28. M. Laxmi, A. Bhardwaj, S. Mehta, and A. Mehta, Development and characterization of nanoemulsion as carrier for the enhancement of bioavailability of artemether, *Artif Cells Nanomed Biotechnol*, **43**, 5 (2015)
29. Preeti, S. Sambhakar, R. Malik, S. Bhatia, A. Al Harrasi, C. Rani, R. Saharan, S. Kumar, Geeta, and R. Sehrawat, Nanoemulsion: An Emerging Novel Technology for Improving the Bioavailability of Drugs, *Scientifica*, (2023)
30. I. A. Wiwiek, S. Martodihardjo, . S., . J., I. G. M. Ngurah Budiana, and . M., Preparation and In-Vitro characterization of Self-Nano emulsifying system of C- Phenylcalix-[4]-Resorcinaryl Octacinnamate and C-Methylcalix-[4]-Resorcinaryl Octabenzoate as ultraviolet absorbers, *Bali Medical Journal*, **6**, 3 (2017)
31. A. Aprilya, R. Rahmadevi, and I. Meirista, Formulasi Nanoemulsi dengan Bahan Dasar Minyak Ikan (Oleum Iecoris Aselli), *Jurnal Sains dan Kesehatan*, **3**, 3 (2021)
32. L. A. Redhita, M. U. Beandrade, I. K. Putri, and R. Anindita, Formula dan Evaluasi Nanoemulsi Ekstrak Daun Kemangi (Ocimum basilicum L.) dengan Variasi Konsentrasi Tween 80, *Jurnal Mitra Kesehatan*, **4**, 2 (2022)
33. A. Indalifiany, M. Hajrul Malaka, A. Fristiohady, R. Andriani, and M. Harul Malaka, Formulasi dan Uji Stabilitas Fisik Nanoemulgel Ekstrak Etanol Spons Petrosia Sp., *Jurnal Farmasi Sains dan Praktis (JFSP)*, 7, 3 (2021)
34. A. C. Adi, C. Christanto, H. Rachmawati, and A. Adlia, Vitamin E-based Folic Acid Nanoemulsion: Formulation and Physical Evaluation for Oral Administration, *Pharm Nanotechnol*, **7**, 4 (2019)
35. D. H. Pham and T. T. Nguyen, Preparation of Tamanu Oil Nanoemulsions by Phase Inversion Temperature, *IOP Conf Ser Mater Sci Eng*, **991**, (2020)
36. H. Sun, Y. Ma, X. Huang, L. Song, H. Guo, X. Sun, N. Li, and M. Qiao, Stabilization of flaxseed oil nanoemulsions based on flaxseed gum: Effects of temperature, pH and NaCl on stability, *LWT*, **176**, (2023)
37. S. M. Jafari and D. J. McClements, Nanoemulsions: Formulation, Applications, and Characterization. London: Academic Press, (2018)
38. B. Ma'arif, R. Azzahara, F. Rizki, A. Suryadinata, A. Wafi, N. Maulina, dan H. Sugihantoro, Formulasi dan Karakterisasi Nanoemulsi Ekstrak Etanol 70% Daun Semanggi (Marsilea crenata C. Presl.), *Medical Sains: Jurnal Ilmiah Kefarmasian*, **8**, 2 (2023)
39. Y. Chang and D. J. McClements, Optimization of Orange Oil Nanoemulsion Formation by Isothermal Low-Energy Methods: Influence of the Oil Phase, Surfactant, and Temperature, *J Agric Food Chem*, **62**, 10 (2014)
40. K. Sridhar, Z. Lou, N. Kalia, A. Najda, A. Bains, and P. Chawla, Woodfordia fruticosa extract nanoemulsion: Influence of processing treatment on droplet size and its assessment for in vitro antimicrobial and anti-inflammatory activity, *Front Nutr.*, **9**, (2022)
41. U. Chasanah, F. Yudastama, and D. Rahmasari, Characteristics and Stability of Candle Nut Oil (Aleurites Moluccana) Nanoemulsion Hair Tonic Preparation, *KnE Medicine*, **2**, 3 (2022)
42. H. Hassanzadeh, M. Alizadeh, R. Hassanzadeh, and B. Ghanbarzadeh, Garlic essential oil-based nanoemulsion carrier: Release and stability kinetics of volatile components, *Food Sci Nutr*, **10**, 5 (2022)
43. U. Rastuti, U. Sulaeman, S. Widyaningsih, S. K. Zia, and R. A. Mahendra, Synthesis and characterization of nanoemulsion of citronella oil (Cymbopogon Nardus L. Rendle) and its Sunscreen Potential Evaluation, *Jurnal Rekayasa Kimia & Lingkungan*, **17**, 1 (2022)
44. U. Rastuti, S. Widyaningsih, M. Chasani, R. K. Habibie, V. F. R. Elly, and S. K. Zia, Formulation, Characterization, and Sunscreen Potential Evaluation of Nutmeg Leaf Essential Oil Nanoemulsions (Myristica fragrans Houtt.), *Molekul*, (2023)
45. U. Rastuti, S. Widyaningsih, M. Chasani, R. K. Habibie, V. F. R. Elly, and S. K. Zia, Formulation, Characterization, and Sunscreen Potential Evaluation of Nutmeg Leaf Essential Oil Nanoemulsions (Myristica fragrans Houtt.), Molekul, (2023)
46. Mardiyanto, R. Mohadi, N. A. Fithri, and G. Kurniawan, Optimization of Nanoemulsion Formula Containing Erythromycin with VCO and Varying Concentrations of Tween-80 and PEG-400, *Science and Technology Indonesia*, (2024)

47. A. Arianto and C. Cindy, Preparation and evaluation of sunflower oil nanoemulsion as a sunscreen, *Journal of Medical Sciences*, **7**, 22 (2019)
48. M. Maharini, R. Rismarika, and Y. Yusnelti, Pengaruh konsentrasi PEG 400 sebagai kosurfaktan pada formulasi nanoemulsi minyak kepayang, *Chempublish Journal*, **5**, 1 (2020)
49. A. I. Widyastuti and D. Saryanti, Formulasi dan Evaluasi Sediaan Nanoemulsi Ekstrak Umbi Bawang Putih (Allium sativum L.), *Jurnal Sains dan Kesehatan*, **5**, 2 (2023)
50. A. N. Aprilya, R. Rahmadevi, and I. Meirista, Formulasi Nanoemulsi dengan Bahan Dasar Minyak Ikan (Oleum Iecoris Aselli*), Jurnal Sains dan Kesehatan*, 3, 3 (2021)
51. G. Ardian, Hajrah, and A. M. Ramadhan, Formulasi Nanoemulsi dari Kombinasi Ekstrak Bunga Mawar (Rosa damascena Mill) dan Ekstrak Bengkoang (Pachyrhizus erosus) dengan Pembawa Minyak Medium Chain Tryglicerides (MCT Oil), *Proceeding of Mulawarman Pharmaceuticals Conferences*, **8**, (2018)
52. M. Khorami, M. Nasr-Esfahani, S. Rahimi, and A. Aarabi, Formation of Nigella Sativa L. seed oil nanoemulsion-based delivery systems by sonication: Factors affecting particle size and stability, *J Am Oil Chem Soc*, (2023)
53. N. N. Aini, Karakteristik Sediaan Nanoemulsi dari Ekstrak Etanol Daun pada Berbagai Tumbuhan:Tinjauan Literatur, SKRIPSI, Universitas Muhammadiyah Magelang, Magelang, (2020)
54. N. N. Indriani, Sintesisn dan Uji Aktivitas Nanoemulsi Ekstrak Etanol Lengkuas Merah (Alpinia purpurata (Vieill) K. Schum) sebagai Antibakteri Klebsiella pneumonia, SKRIPSI, Universitas Islam Indonesia, Yogyakarta, (2020)
55. T. Imanto, R. Prasetiawan, and E. R. Wikantyasning, Formulation and Characterization of Nanoemulgel Containing Aloe Vera L. Powder, *Pharmacon: Jurnal Farmasi Indonesia*, 16, 1 (2019)
56. I. Bhusan Basumatary and S. Kumar, Effect of Surfactant Concentration on Physicochemical and Antibacterial Properties of Eugenol Nanoemulsions, *Current Indian Science*, **02**, (2024)
57. M. K. Anwer, S. Jamil, E. O. Ibnouf, and F. Shakeel, Enhanced Antibacterial Effects of Clove Essential Oil by Nanoemulsion, *J Oleo Sci*, **63**, 4 (2014)