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Fabrication, Characterization, and Toxicity Assay of Nanoemulsions from *Tagetes erecta* and *Dillenia serrata* Thunb. Extracts

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Nature-based medicine has emerged to reduce the adverse effects of the therapeutic techniques primarily used today. However, the use of natural products often results in suboptimal pharmacological effects due to their poor permeability and solubility. Nanoemulsion is an emerging drug delivery technology that improves bioactive compounds' physical and chemical properties in *in vitro* and *in vivo* assays. Leaf extracts of Dengen (*Dillenia serrata* Thunb.) and Kenikir (*Tagetes erecta*) were mixed with ethanol, Tween 80, PEG 400, virgin coconut oil (VCO), and phosphate buffer in an ultrasonicator probe to fabricate nanoemulsions. Physical and chemical analyses were employed to characterize the nanoemulsions. Toxicity assay was also conducted to analyze the pharmacological effect. The nanoemulsions of Dengen and Kenikir leaves have a yellow to brownish color, distinctive aroma, and homogeneous and runny texture. Stability test indicated that all formulations maintained their integrity and did not separate over time, confirming their thermodynamic stability. All formulations were O/W type with emulsification times of 8.21 to 11.71 seconds and soluble in polar solvents like methanol. Both nanoemulsions' characteristics met the specified particle size range of 24.66 to 40.55 nm, with a polydispersity index of 0.35 to 0.36 and a zeta potential of -8.47 to -4.98 mV. Other characteristics include a viscosity of 6.04 to 6.34 cP, a density of 1.02 g/cm^3, a range of pH values of 5.1 to 5.7, and a transmittance percentage of 92.9 to 98.6%. Both the Dengen and Kenikir nanoemulsions were highly toxic to *Artemia salina* Leach larvae with IC_{50} of 0.0106 and 0.0098 ppm, respectively. These results indicate that the leaf extract nanoemulsions' physical and chemical properties provide prospective pharmacological effects.

Keywords: Herb; medicine; nanoemulsion; toxicity

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Plant extracts are a source of secondary metabolites used in various pharmacological activities to treat multiple diseases. According to a previous study, two traditional Indonesian plants used for medication are *Dillenia serrata* Thunb. (Dilleniaceae) and *Tagetes erecta* (Asteraceae). Locally, *D. seratta* Thunb., which is endemic to Sulawesi [1], is known as Dengen, whereas *Tagetes erecta* is known as Kenikir. The plants are used as remedies for mouth ulcers, fever, vomiting blood, wound [2], renal problems, indigestion, muscle cramps, ulcers, and hemorrhoids [3, 4]. Dengen additionally demonstrates pharmacological properties such as anti-inflammatory, antioxidant, antibacterial, and anticancer [2]. Dengen leaf extract contains numerous secondary metabolites, namely triterpenoids, alkaloids, saponins, flavonoids, and polyphenols [5]. While Kenikir contains quinones, saponins, steroids, terpenoids, anthocyanins, betacyanins, coumarins, flavonoids, glycosides, phenolics, and tannins [6]. However, using natural products as medications comes with drawbacks, such as poor permeability and solubility. Because of the resulting limited bioavailability, therapeutic use necessitates large doses [7]. Therefore, an appropriate drug delivery method is needed to address the problems with the solubility and bioavailability of the chemicals present in plant extracts.

Nanotechnology is one of the disciplines of science that is currently expanding the fastest, especially in the medical industry. The most rapidly progressing application of nanotechnology in the health industry is the development of new drug delivery systems that encapsulate active plant metabolites in biodegradable and biocompatible nanomaterials [8]. Nanoemulsions have been shown through both in *in vitro* and *in vivo* analyses to improve the stability and bioavailability of bioactive compounds in pharmaceuticals [9]. Samsiar et al. (2021) define nanoemulsion as a transparent, stable emulsion system with petite particle sizes (20-200 nm) [10]. Nanoemulsions are composed of a mixture of oil, surfactant, cosurfactant, and aqueous phases, which can be prepared using high-energy methods such as high-pressure homogenization, ultrasonication, and microfluidisation, as well as low-energy methods such as phase inversion methods [11].

Extract preparation into nanoemulsions aims to increase the absorption efficiency in the body to extend the activity [12]. In this study, the leaves of Dengen and Kenikir would be prepared into nanoemulsions that can later be used to develop new drugs with high solubility and bioavailability.

EXPERIMENTAL

Chemicals and Materials

The materials used in this study were Dengen (*D. serrata* Thunb.) leaves, Kenikir (*T. erecta*) leaves, sodium dihydrogen phosphate (NaH_2PO_4, Merck), disodium hydrogen phosphate (Na_2HPO_4, Pudak), virgin coconut oil (VCO, Trivico), Tween 80 (Brataco), PEG 400 (Brataco), ethanol (Merck), and distilled water.

Sample Extraction

Samples of Dengen and Kenikir leaves were extracted using the maceration process. The leaves of both plants were soaked in an ethyl acetate solution because it has a medium polarity with minimal cell toxicity [13]. The extraction was carried out in a confined area for 3 x 24 hours at room temperature and controlled by the TLC method. A rotary evaporator was connected to the resultant filter to obtain a thick extract.

Formulations of Dengen and Kenikir Leaf Extract Nanoemulsions

The nanoemulsion was fabricated by combining leaf extract, ethanol 96%, Tween 80, PEG 400, and VCO in accordance with the formulation and stirred for 30 minutes using a hotplate stirrer. Phosphate buffer was then added to 100 grams and mixed for 30 minutes. The emulsification process was enhanced for one hour by ultrasonic sonication using an ultrasonic probe [14]. The nanoemulsions were made using the formulations presented in Table 1. The concentration of the extract was used as the independent variable in this study.

Nanoemulsion Stability Test

The physical stability of the nanoemulsions was tested through a centrifugal test at 3000 rpm for 75 minutes to see whether or not phase separation was present [15]. Nanoemulsion stability tests were also conducted to evaluate the thermodynamic stability. The nanoemulsion formulations were stored at a low temperature of 4°C and continued at a high temperature of 40°C for 24 hours. This treatment was conducted in two cycles and observed organoleptically and measured pH [7].

Table 1. Formulations for Dengen and Kenikir Leaf Extract Nanoemulsions.

Materials	Formulation				Function
	I	II	III	IV	
Extract (g)	0.01	0.05	0.08	0.1	Active matter
VCO (g)	1	1	1	1	Oil
Tween 80 (g)	7	7	7	7	Surfactant
PEG 400 (g)	3	3	3	3	Cosurfactant
Ethanol 96% (g)	2	2	2	2	Cosurfactant
Phosphate buffer (g)	add to 100				Solvent

Characterization of Nanoemulsions

Organoleptic Test

Nanoemulsion organoleptical test was carried out by the panel by observing color, aroma, solidity, and homogeneity [16].

Nanoemulsion Type Test

Nanoemulsion type test was carried out using the dilution method. Nanoemulsion samples were added to distilled water in a ratio of 1:50. If a nanoemulsion is soluble in distilled water, then the nanomulsion belongs to the type of oil in water [7].

Emulsification Time Test

Emulsification time was evaluated by dripping 1 mL of the formula into 50 mL of distilled water and mixed using a hotplate stirrer at a speed of 100 rpm. The calculation starts from the beginning of the drop down until emulsifies perfectly into the distilled water [17].

Solubility Test

Solubility test was carried out by mixing the nanoemulsion in a 100 mL glass containing an organic solvent (1:1); the organic solvents used were of different levels of polarity, which were *n*-hexane, ethyl acetate, and methanol [18].

Viscosity and Density Measurements

Viscosity test was performed using a viscometer. The nanoemulsion is placed in a glass container, and then the spindle is lowered until the edge of the spindle is immersed in the sample. Meanwhile, density was measured using a pycnometer with distilled water as standard [19].

Measurement of pH and Transmittance

The pH of the nanoemulsion extracts was measured using a pH meter. Prior to use, the electrode is calibrated using standard pH 4 and 7 suspension solutions. The calibration process is complete when the displayed pH meets the buffers' standard and stable pH values. After that the electrode is immersed into the preparation, the pH value will be displayed on the screen [16]. Transmittance was measure using a UV-Vis spectrophotometer. 100 µL of the nanoemulsion sample is dissolved in 5 mL of distilled water and homogenized by vortexing for 1 minute and transmittance is measured at the wavelength of 650 nm [7].

Tests on Particle Size, Polydispersity Index, and Zeta Potential

All tests were performed using a particle size analyzer (PSA). A total of 10 mL of the preparation is taken and inserted into the cuvette. The cuvette must be cleaned first as not to affect analysis results. The cuvette filled with the nanoemulsion is then inserted into the sample holder and analyzed by the instrument [16].

Toxicity Assay

Artemia salina Leach shrimp eggs were hatched in aerated artificial seawater (salinity 38 g/L), incubated under a light bulb. The larvae used for analysis were 48 hours old [20]. The nanoemulsions of Dengen and Kenikir leaf extracts were diluted with artificial seawater and 15 shrimp larvae were added to obtain the final concentrations of 3.10^{-1}, 3.10^{-2}, 3.10^{-3}, and 3.10^{-4} ppm. The series of solutions containing shrimp larvae were then incubated for 24 hours under a light bulb. After incubation was complete, the mortality rate (%) of shrimp larvae at each concentration was calculated and converted into probit values. This analysis procedure generally refers to Meyer et al. (1982), with slight modifications [21].

RESULTS AND DISCUSSION

Nanoemulsions of Dengen and Kenikir Leaf Extracts

Nanoemulsions of Dengen and Kenikir leaf extracts were fabricated using virgin coconut oil (VCO), Tween 80, PEG 400, ethanol, and phosphate buffer pH 6. VCO was chosen as a carrier oil because it is not easily oxidized, has good solubility, and can produce droplet size < 100 nm [22]. Tween 80 was added to the nanoemulsions as a surface agent, which helps to form a single layer and reduces the surface tension between the water and oil layers. Tween 80 is a hydrophilic surface agent with heterogeneous oxygen atoms, so it is anticorrosive and has low toxicity. To stabilize the layer on the globules and increase the consistency of the globule diameter, PEG 400 and 96% ethanol were used as cosurfactants [23]. Phosphate buffer was employed as a water phase in the synthesis of the nanoemulsions.

Nanoemulsion synthesis was done using high-energy methods, one of which was ultrasound. This method provides powerful mechanical energy to create nano-sized particles. Ultrasonication was chosen because it is easier to operate and a clean system. This method uses an ultrasonic probe capable of emitting ultrasonic waves to break macroemulsions into nanoemulsions due to fluctuations in the pressure of aquatic waves [24]. The results of the nanoemulsion synthesis are shown in Figure 1.

The results of the nanoemulsion synthesis show that there were differences in color intensity among the nanoemulsions. The higher the concentration of the extract, the more intense the color. These results are in line with the research of Ma'arif et al. (2023), nanoemulsions of Semanggi (*Marsilea crenata* C. Presl.) leaf extracts produce different color intensities due to differences in extract concentration [7]. In addition, the effect of the sonication process on nanoemulsion particles produces a homogeneous nanoemulsion because the cavitation effect produced by ultrasonic waves causes the rupture of molecules in the nanoemulsion, resulting in the same molecular size [19].

Stability Test

According to Redhita et al. (2022), the centrifugation stability test of nanoemulsions describes the impact of Earth's gravitational pull on the resilience of the nanoemulsion supply in storage for a year [25]. The results indicate that following centrifugation tests, none of the nanoemulsion formulations exhibited phase separation, sedimentation, or creaming. The application of the non-ionic surfactant Tween 80 has an impact on these results. By creating a film layer on the droplet surface, Tween 80 inhibits aggregation and droplet coalescence in the dispersion medium [26]. Likewise, Dista et al. (2022) demonstrated the same outcomes, showing that the nanoemulsion of Sungkai leaf extract made with VCO, Tween 80, and PEG 400 was physically stable because it did not exhibit phase separation, creaming, or precipitation [27].

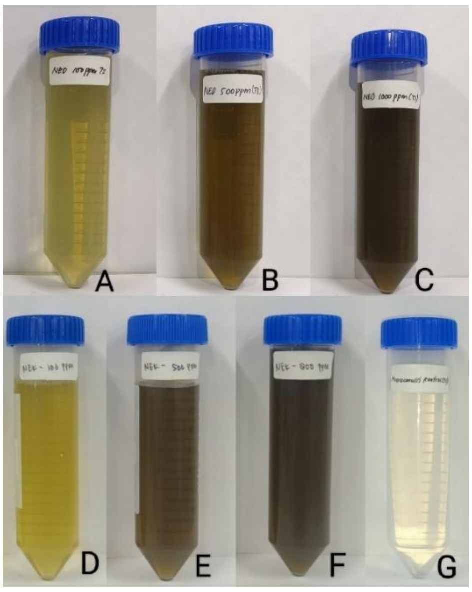

Figure 1. Dengen and Kenikir nanoemulsions. (A) NED 100 ppm; (B) NED 500 ppm; (C) NED 1000 ppm; (D) NEK 100 ppm; (E) NEK 300 ppm; (F) NEK 800 ppm; and (G) control.

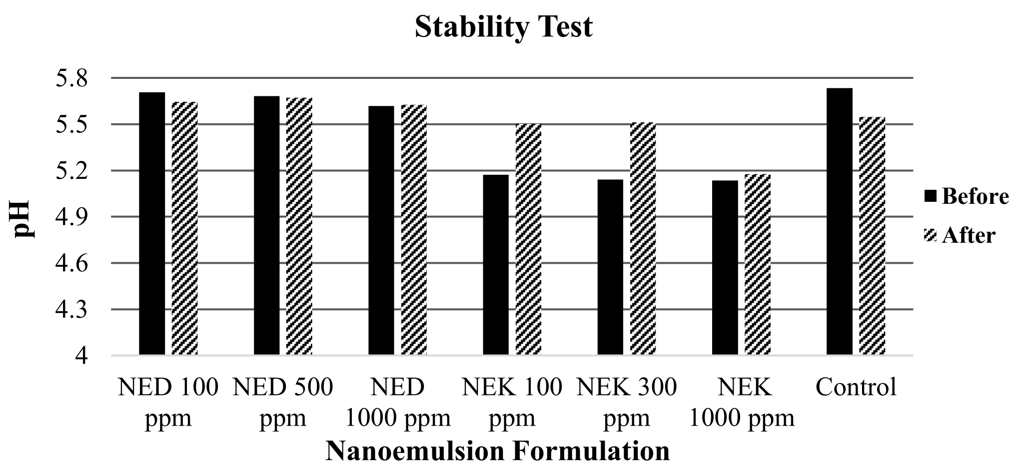

Figure 2. Comparison of pH values before and after cycling test.

pH measurement was used to ascertain whether or not pH change occurred prior to and following the cycling test. The results are displayed in Figure 2. The test results reveal that all the nanoemulsion formulations displayed no significant differences, indicated with P-value > 0.05. Similar findings were reported in a study by Widyastuti and Saryanti (2023), which found that after cycling tests, garlic bulb extract nanoemulsions made with VCO, Tween 80, and PEG 400 did not significantly change in pH [28]. These findings demonstrate the stability of nanoemulsions in the face of drastic temperature changes. According to Ma'arif et al. (2023), a change in pH signifies the ionization or degradation of the nanoemulsion's component composer [7].

Characterization

Organoleptic Test

Based on the tests carried out, the higher the concentration of the extract, the more concentrated color is produced, ranging from uncolored to brown. The aroma of the Dengen nanoemulsions remained unaffected by the quantity of the extract added. All the Dengen formulations smelled of typical Tween 80. On the other hand, there was a slight difference in the Kenikir nanoemulsions. The Kenikir nanoemulsion with the concentration of 100 ppm had a typical aroma of Tween 80, while the concentrations of 500 and 800 ppm smelled more like extracts. Every formulation was runny and did not exhibit homogeneity or phase separation. Organoleptic test of nanoemulsion aims to determine the physical differences between nanoemulsion formulations, which include color, aroma, homogeneity, and texture. Based on the results of the nanoemulsion organoleptic test (Table 2), all the nanoemulsion formulas were transparent, runny, and homogeneous. According to Destiyana et al. (2018), nanoemulsion parameters are good if there is no phase separation, homogeneous, and transparent [16]. In addition, the test results show that there were differences in color intensity and aroma between the nanoemulsion formulations. The higher the concentration of the extract, the more intense the color produced, ranging from colorless to brown [7]. The aroma produced in the Dengen nanoemulsions had a distinctive aroma of Tween 80, while in the Kenikir nanoemulsions there was a slight difference. The Kenikir nanoemulsion with the concentration of 100 ppm had a distinctive aroma of Tween 80, while the concentrations of 500 and 800 ppm were more of the flavorful extracts. According to Zen et al. (2020), Kenikir plant is known as a plant that has a very pungent distinctive aroma, this is what that causes the difference in the aroma of the Kenikir nanoemulsion preparations at the concentrations of 500 and 800 ppm produced [29]. The organoleptic test results align with Widyastuti and Saryanti's (2023) research on the organoleptic parameters of garlic bulb nanoemulsion extracts, which were lymphatic yellow in color, distinctive odor, clear, and homogeneous [28].

Nanoemulsion Type Test

Nanoemulsions are grouped into several types based on the chemical properties of their dispersed phases. Oil-in-water (O/W) is a type of oil-dispersed nanoemulsion in the water phase, whereas water-in-oil (W/O) is the type when water is dispersed into the oil phase. Both types are grouped as single nanoemulsions. In the meantime, there are multiple nanoemulsions with more complex arrangements. These nanoemulsions are made by dispersing a single nanoemulsion in another liquid phase [30].

The results show that all the nanoemulsion formulations were perfectly dispersed in distilled water, meaning that the synthetic nanoemulsions are oil-in-water-type nanoemulsions, similar to that reported by Indalifiany et al. (2021) [14]. The results are consistent with the theory because the combination of surface agents and co-surfactors has a hydrophile-lipophile balance (HLB) value of more than 8. According to Smail et al. (2021), if the HLB value is between 8 to 18, then an oil-in-water nanoemulsion will form [31].

Table 2. Results of the nanoemulsion organoleptic test.

Formulation of Nanoemulsion	**Parameters**			
	Color	**Aroma**	**Homogeneity**	**Texture**
NED 500 ppm	Brownish-yellow	Typical Tween 80	Homogeneous	Runny
NED 1000 ppm	Brownish-yellow	Typical Tween 80	Homogeneous	Runny
NEK 100 ppm	Light yellow	Typical Tween 80	Homogeneous	Runny
NEK 500 ppm	Yellow	Typical extracts	Homogeneous	Runny
NEK 800 ppm	Brown	Typical extracts	Homogeneous	Runny
Control	Colorless	Typical Tween 80	Homogeneous	Runny

Emulsification Time Test

Emulsification time testing aims to determine how long it takes for the nanoemulsion formula to emulsify spontaneously without any significant shaking process. The requirement for a better emulsification time is less than two minutes [32]. From the research conducted by Daryati et al. (2022), the emulsification time required by nanoemulsions of African leaf (*Vernonia amygdalina*) extracts was 2.34 to 3.37 seconds [33]. The research by Rusminingsih et al. (2023) also reported that the emulsification time required by Moringa (*Moringa oleifera* Lam) leaf nanoemulsions was 5.9 to 19 seconds [34].

The emulsification time test results are shown in Table 3. The time required by all the nanoemulsion formulations and the control ranged from 8.21 to 11.71 seconds. The test results show that all the nanoemulsions could emulsify in less than two minutes. The emulsification time of less than two minutes indicates that the nanoemulsion formulation is able to form an emulsion system when inside the body by producing a clear emulsion system. In contrast, if the emulsification time is more than two minutes, it will produce a cloudy emulsion system that reduces the absorption rate of the nanoemulsion in the body [35]. According to Sahumena et al (2019), a short emulsification time is influenced by the cosurfactants and surfactants used. These two components form the interface layer between the oil and water phases. The presence of cosurfactants will form an empty space between surfactants, causing the preparation to have high fluidity and be able to emulsify quickly [36].

Solubility Test

The solubility of a substance is affected by the polarity of the solvent. Solubility is an important parameter for reviewing the absorption ability of nanoemulsion preparations into the body. The solubility of a nanoemulsion is in line with its bioavailability. Preparations with high solubility can be absorbed into the circulatory system so as to produce optimal therapeutic effects [37]. According to Zola et al. (2023), a drug with low solubility in water is often difficult to absorb in the body, so the preparation's solubility must be increased [38]. Tests on the solubility of the nanoemulsions of the Dengen and Kenikir leaf extract were carried out using three types of solvents with different polarities: methanol, ethyl acetate, and *n*-hexane.

The nanoemulsions were soluble in methanol, exhibiting a polar character. Based on this, the solubility of the nanoemulsion preparations in methanol as a polar solvent shows that the nanoemulsions could be able to be absorbed by the body. The results obtained in this study are in line with the research by Jusnita et al. (2019), who reported that the solubility test of Peridot leaf extract nanoemulsion using solvents with different polarity levels (*n*-hexane, ethyl acetate, and methanol) showed that the extract nanoemulsion dissolved perfectly in methanol as a polar solvent [18]. Also, the research by Nasiro et al. (2023) conducted solubility tests on Temulawak extract nanoemulsion and the results obtained showed that the nanoemulsion was soluble in polar solvents (methanol, ethanol, and water) [39].

Viscosity and Density Measurements

The nanoemulsions of the Dengen and Kenikir leaf extracts were measured for viscosity and density. Viscosity is one of the important parameters to measure the stability of a nanoemulsion. Viscosity is the ability of a fluid to withstand a given force. According to Widyastuti and Saryanti (2023), the viscosity value of a good nanoemulsion preparation is 1 to 100 cP [28]. The density test of the nanoemulsions was carried out using a pycnometer. The specific gravity value is expressed in decimal form. The results of viscosity and density measurements of the nanoemulsions are shown in Table 4.

Table 3. Emulsification times of nanoemulsions.

Formulation of Nanoemulsion	Emulsification Time (s)
NED 100 ppm	8.21
NED 500 ppm	10.19
NED 1000 ppm	11.71
NEK 100 ppm	9.23
NEK 500 ppm	7.33
NEK 800 ppm	8.94
Control	8.59

Table 4. Results of viscosity and density measurements of nanoemulsions.

Formulation of Nanoemulsion	Viscosity (cP)	Density (g/cm^3)
NED 100 ppm	6.06	1.02
NED 500 ppm	6.04	1.02
NED 1000 ppm	6.04	1.02
NEK 100 ppm	6.26	1.02
NEK 500 ppm	6.34	1.02
NEK 800 ppm	6.18	1.02
Control	6.18	1.02

Based on Table 4, the viscosity values of the nanoemulsions obtained were in the range of 6.04 to 6.42 cP, and these values still meet the requirements for the viscosity value of nanoemulsion. The viscosity values of five nanoemulsion formulations fall into the low viscosity category. According to Shabrina and Khansa (2022), low viscosity value is due to nanoemulsions belonging to the oil-in-water type, with the amount of water phase being more than 70% [40]. Low viscosity value will allow the spread of active substances to occur evenly into the body. According to Herbianto (2018), the density of a nanoemulsion is influenced by the amount of surfactant used; the more surfactant there is, the higher the density of the nanoemulsion [41]. In this study, the density of all the nanoemulsion formulations produced have the same value of 1.02 g/cm^3. This is because the amount of surfactant used in all formulations was the same. The results of the viscosity and density measurements of the nanoemulsion preparations carried out are comparable to the research conducted by Ayuningtyas et al. (2022), which reported that the nanoemulsion mouthwash of the ethanol extract of Jeringau (*Acorus calamus* Linn.) leaves has a relatively low viscosity but still meets the requirements of the viscosity of 1.47 to 1.68 cP and the density of 1.026 to 1.030 g/cm^3 [42].

Measurement of pH and Transmittance

The pH of the nanoemulsions of the Dengen and Kenikir leaf extracts was tested using a pH meter. The pH of the nanoemulsions could be maintained in the desired pH range by using phosphate buffer solution (pH 6) as the water phase. The research by Jusnita and Nasution (2019) reported that using phosphate buffer solution as the water phase could maintain the pH of the nanoemulsion produced [43]. The pH test was conducted to determine whether the synthesized nanoemulsions meet the range of pH values that are safe and acceptable to the body. Based on the results of the pH test, all the nanoemulsion formulations of the Dengen and Kenikir extracts were in the pH range of 5.140-5.734, which is in the pH range that is safe for the skin; in the pH range of 4 to 6.5, so that it does not irritate when applied [44][25]. In this study, Tween 80, which is a non-ionic surfactant, was used in the same amount for all the nanoemulsion formulations, so it did not affect the pH of the nanoemulsions to be more acidic or too basic. According to Ma'arif et al. (2023), pH measurement is one of the important parameters in nanoemulsion synthesis results because each change in pH affects the stability of the properties of each nanoemulsion formulation [7]. The results of this pH test are in accordance with the research of Widyastuti and Saryanti (2023) who synthesized garlic bulb nanoemulsion with VCO, Tween 80, and PEG 400 and found that the pH of the nanoemulsion was in the range of 4.9-5 [28]. The results of the pH measurement of the nanoemulsions are shown in Table 5.

Table 5. pH values of nanoemulsions.

Formulation of Nanoemulsion	pH
NED 100 ppm	5.707
NED 500 ppm	5.683
NED 1000 ppm	5.618
NEK 100 ppm	5.172
NEK 500 ppm	5.140
NEK 800 ppm	5.134
Control	5.734

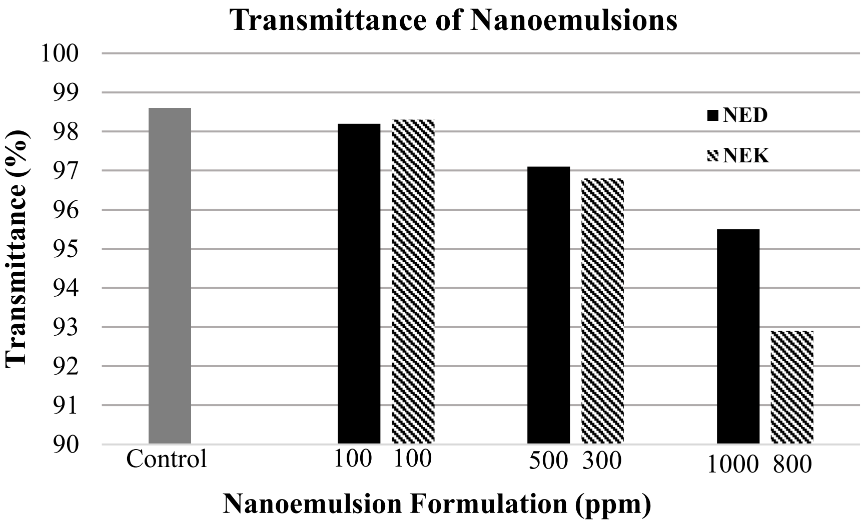

Figure 3. Transmittance values of nanoemulsions.

The percent transmittance test of nanoemulsion was performed to determine the clarity of the nanoemulsions. The test was performed using a UV-Vis spectrophotometer (650 nm). According to Daud et al. (2017), a stable nanoemulsion has a transmittance value of 90 to 100% [15]. The clarity of a nanoemulsion is influenced by the particle size of the nanoemulsion that has reached nanometer size, thus the solution will look transparent and the resulting transmittance is higher [36]. Based on the tests conducted, it was established that all the nanoemulsion formulations have the percent transmittance value in accordance with the requirements, in the range of 92.9 to 98.6%. The test results showed decreases in the percent transmittance of the nanoemulsions with increases in extract concentration. The increase in extract concentration is related to the distribution of nanoemulsion particle size, which can be shown by the difference in color intensity of the nanoemulsion preparations, as shown in Figure 1. A larger particle size of the nanoemulsion will disperse light more strongly, thus reduces the transmittance value. The results obtained are in line with the research conducted by Dista et al. (2022), where decreases were recorded in the percent transmittance of the nanoemulsions of Sungkai leaf extract synthesized using three different extract concentrations; the percent transmittance values obtained were 93.1% (0.26 g), 95.5% (0.4 g), and 98.5% (0.53 g) [27]. The transmittance results of the nanoemulsions are shown in Figure 3.

Tests on Particle Size, Polydispersity Index, and Zeta Potential

Particle size tests were performed to ensure that the synthesized nanoemulsions were nanometer-sized. Based on Table 6, the particle size of the prepared nanoemulsions is in accordance with the nanoemulsion size requirement of 20 to 200 nm [10]. The polydispersity index (PI) value of the nanoemulsions was also tested. PI value indicates the homogeneity or uniformity of the nanoemulsion particle size. According to Zubaydah et al. (2023), a good polydispersity index value is <0.5 [19]. A low polydispersity indicates that the nanoemulsion preparation is monodisperse [40]. Based on Table 6, the PI values of the prepared nanoemulsions were <0.5, namely 0.35 to 0.36. This indicates that all the nanoemulsion formulations meet the particle size distribution standards as nanoemulsion systems. According to Gul et al. (2022), the polydispersity index value is associated with the ability of nanodroplets to be absorbed into the body [44]. The lower the PI value of the nanoemulsion, the higher the permeation of the preparation through the epidermal and dermal layers of the skin.

Zeta potential is an indicator of the magnitude of the repulsion between charged droplets in the disperse system and indirectly indicates the stability of the nanoemulsion dispersion [45]. Nanoemulsions with potential zeta values greater than +30 mV and less than -30 mV are considered highly anionic and cationic [46]. Nanoemulsions with potential zeta values above +30 mV and below -30 mV indicate a more stable preparation and can also prevent agglomeration due to the repulsive force between particles with the same charge [46][47]. Zeta potential measurements were carried out on NED 1000 ppm and NEK 800 ppm nanoemulsion formulations, and the values obtained were -8.47 and -4.98 mV, respectively. The results obtained indicate that the nanoemulsions have a low charge, making them susceptible to flocculation. However, zeta potential is not the main parameter of nanoemulsions' stability, and other factors that are also considered are size, distribution, and morphology [48].

Table 6. Particle size and polydispersity index of nanoemulsions.

Formulation of Nanoemulsion	Result	
	Particle Size (nm)	Polydispersity Index
NED 100 ppm	27.41 ± 0.17	0.36 ± 0.01
NED 500 ppm	24.66 ± 0.12	0.35 ± 0.00
NED 1000 ppm	25.28 ± 0.14	0.36 ± 0.04
NEK 100 ppm	26.12 ± 5.29	0.35 ± 0.02
NEK 500 ppm	29.42 ± 1.34	0.35 ± 0.01
NEK 800 ppm	40.55 ± 5.81	0.36 ± 0.03

Table 7. Toxicity of NED and NEK leaf extracts on *A. salina* L. larvae.

Concentration (ppm)	Log Concentration	NED		NEK	
		Larval Death (%)	Probit	Larval Death (%)	Probit
3.10^{-1}	-0.523	93	6.48	93	6.48
3.10^{-2}	-1.523	60	5.25	53	5.08
3.10^{-3}	-2.523	33	4.56	33	4.56
3.10^{-4}	-3.523	7	3.52	7	3.52
LC_{50} (ppm)		0.0106		0.0098	

Toxicity Assay

The toxicity and larval mortality values are shown in Table 7. The toxicity analysis showed LC_{50} values of 0.0106 and 0.0098 ppm for the nanoemulsions of the Dengen and Kenikir extracts, respectively. Comparing both nanoemulsions to doxorubicin, which has an LC_{50} value of 5.63 ppm, reveals enhanced activities. Doxorubicin, a common chemotherapeutic treatment for cancer, causes cell death by a variety of intracellular interactions, producing reactive oxygen species and DNA-adducted topologies that trigger histone eviction, topoisomerase II inhibition, and apoptosis [49][50]. The toxicity value was included in the active category (LC_{50} < 100 ppm). Emulsions of active compounds from Dengen and Kenikir leaf extracts in nano size with O/W type facilitate the adsorption process of the compounds, therefore they have good biological activities. The toxicity values obtained indicate that the nanoemulsions of the two extracts have the potential to be applied further as an anticancer. In addition, this toxicity data can provide an overview of other potential bioactivities, such as enzyme inhibitors.

CONCLUSION

The nanoemulsions of Dengen and Kenikir leaves have been synthesized and characterized. To optimize the emulsification process to nano-sized, substances including VCO, Tween 80, PEG 400, 96% ethanol, and a high-energy phosphate buffer were mixed and prepared via the method of ultrasonication following the nanoemulsions' formulations. The nanoemulsions were thermodynamically stable and stable for a storage period of one year. All formulations were soluble in polar solvents, such as methanol, and were of oil-in-water-type, with emulsification period ranging from 8.21 to 11.71 seconds. The leaf nanoemulsions of Dengen and Kenikir have met the required particle size with the size range of 24.66 to 40.55 nm, zeta potential of -8.47 to -4.98 mV, and polydispersity index of 0.35 to 0.36. Viscosity of 6.04 to 6.34 cP, density of 1.02 g/cm^3, pH of 5.1 to 5.7, and transmission percentage of 92.9 to 98.6 are some of the other properties. The toxicity assay reveals the future medicinal potential of the nanoemulsions of Dengen and Kenikir extracts with LC_{50} values of 0.0106 and 0.0098 ppm, respectively. These findings suggest that the nanoemulsions of Dengen and Kenikir extracts could serve as effective candidates for developing new therapeutic agents with enhanced bioavailability.

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