



# Acute Toxicity Nanoemulsion of Ethanol Extract of Clove Leaves (*Syzygium aromaticum* L.)

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**Abstract:** Clove leaves have been widely used as traditional medicine. Clove leaves are rich in saponins, flavonoids, tannins, sterols, and essential oils. To improve the function of clove leaves, one of them is by reducing the sample size into nanoemulsion form. Nanoemulsions were prepared using the sonication method and exhibited an average droplet size of 13.3 nm. The purpose of this study was to determine the toxic properties, the dose that can cause toxic effects, and the LD50 value of nanoemulsion administration of ethanol extract of clove leaves. This study used 25 male white rats which were divided into five groups, namely a control group (nanoemulsion base) and four treatment groups receiving oral doses of 5, 50, 300, and 2000 mg/kg BW. The LD50 value was calculated using AOT425 StatPgm in accordance with OECD TG 425 (Up and Down Procedure). Mortality summary: control 0/5; 5 mg/kg BW 0/5; 50 mg/kg BW 0/5; 300 mg/kg BW 0/5; 2000 mg/kg BW 5/5. The results showed an LD50 of 798.5 mg/kg BW and, based on the GHS, it falls under Acute Toxicity (Oral) Category 4 (300–2000 mg/kg BW). Clinical signs such as piloerection, grooming, and weakness appeared mainly in the early observation phase (from 30 minutes to 4/8/12 hours) in each dose group and were reversible.

**Keywords:** Clove leaves; Lethal dose (LD50); Nanoemulsion; Toxicity

## Introduction

Indonesia has a natural ecosystem with abundant biodiversity, so it is included in natural areas with high biodiversity (Qoriasmadillah et al., 2024). Most of the use of drugs in Indonesia is still processed by traditional methods and is still a custom or habit of a society (Yeni et al., 2025). The use of traditional medicine attracts the attention of the public, both academics and health professionals, to find out the level of safety, the benefits of using a plant with medicinal properties (Mustapa, 2018). The exploration of plants extracts for bioactive compounds continues to expand into various therapeutic applications (Maulidza et al., 2025). One of the plants in Indonesia as a traditional medicine is clove

leaves (*Syzygium aromaticum* L.) (Kamaruddin, 2021). Clove leaves have antiviral, antimicrobial, antifungal, anticancer, and anti-inflammatory activities from the active content of clove leaves, namely eugenol (Batiha et al., 2020). Phytochemical studies have confirmed that clove leaf and flower extracts contain antioxidant compounds (Lumingkewas et al., 2023). Clove leaves are rich in saponins, flavonoids, tannins, sterols, and essential oil content of 1-4% which acts as a natural antioxidant (Kuncoro et al., 2023). In addition, clove leaf oil contains phenolic compounds such as eugenol, eugenol acetate, caryophyllene and sesquiterpenes, which are believed to have aphrodisiac properties that can increase arousal (Tuldjanah et al., 2023). Eugenol have low solubility and poor bioavailability, therefore

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the solution is to reduce the sample size to nano shape (Ramadhani, 2022).

Some types of nanotechnology development are nano-medicine, nanoparticles, and nanoemulsions. According to Mahiya et al. (2022). Plant extract nanoemulsions have been developed for various therapeutic purposes including wound healing (Nasution et al., 2023). Nanoemulsion can be one of the delivery system options that can improve the solubility and bioavailability of oral administration (Chiuman et al., 2024). Nanoemulsion is an emulsion system that is transparent and is a combination of water-oil stabilized by surfactant molecules measuring 50-500 nm. The advantages of nanoemulsions are that they are energy-free and have a larger surface area, making it easier and faster to interact with targets (Nasiro et al., 2023). The development of nanoemulsion formulas in order to be used in medicine must go through various tests, one of which is toxicity tests (Sandhiutami et al., 2022). Particle size evaluation using Particle Size Analyzer (PSA) and zeta potential measurement are critical parameters in characterizing nanoemulsion systems (Sari et al., 2024).

A plant compound can be considered safe if it passes animal testing and is clinically safe for consumption, therefore, it is very important to know the LD50 and the spectrum of toxic effects (Merlani et al., 2024). Acute toxicity tests aim to detect the presence of toxicity in a substance or material obtain hazard data after being given an acute dose of a compound (Kamaruddin, 2021). Acute toxicity tests using experimental animals are necessary to detect toxic effects that appear shortly after administration of a substance in a single dose or repeated doses administered within no more than 24 hours (BPOM, 2022). Based on the background and literature on clove leaves, it is necessary to conduct research on the acute toxicity test given by nanoemulsion of clove leaf ethanol extract to determine the toxicity of nanoemulsion of clove leaf ethanol extract showing toxic properties and determining the value of acute toxicity potential (LD50) after the administration of nanoemulsion of clove leaf ethanol extract (*Syzygium aromaticum* L.) metabolites based on their group using the OECD 423 method

## Method

### Materials

Mesh sieve 40, stirring rod, maceration vessel, blender, porcelain cup, beaker, measuring cup, watch glass, scissors, cloth test animal cage, magnetic stirrer, water bath, droppipette, rotary vacuum evaporator, horn spoon, 3 ml oral sonde, 1 ml injection spoit, spot plates, markers, gram scales, analytical scales and containers. Distilled water, aluminum foil, 2 N hydrochloric acid, anhydrous acetic acid, Dragendorff,

Ethanol 96%, FeCl<sub>3</sub>, HCl, concentrated H<sub>2</sub>SO<sub>4</sub>, cotton, mask, NaOH 10%, plastic wrap, gloves, clove leaf nanoemulsion preparation (*Syzygium aromaticum* L) and 25 male white rats.

### Detailed Procedure

#### Extract Manufacturing

Simplicia powder is extracted by maceration using 96% ethanol solvent (3x24 hours). Simplicia powder is weighed as much as 1,000 grams. The extract can be concentrated with a Rotary Evaporator until a thick extract is obtained. Then a phytochemical filtration test is carried out to detect the presence of secondary metabolites based on their group and also as preliminary information to determine the class of chemical compounds that have biological activity from a plant in the form of simplicia or extract. Then a phytochemical filtration test is carried out to detect the presence of secondary metabolites based on their group and also as preliminary information to determine the class of chemical compounds that have biological activity from a plant in the form of simplicia or extract.

#### Making of Nanoemulsions

Nanoemulsion of ethanol extract of clove leaves was prepared using sonication method with Tween 80 as surfactant, PEG 400 as co-surfactant, and virgin coconut oil (VCO) as oil phase (Putri et al., 2024). This method has been widely used for formulating nanoemulsions from plant extracts to achieve droplet sizes below 100 nm (Dewi et al., 2022). Tween 80 is mixed with propylene glycol using a magnetic stirrer at a speed of 500 rpm. After homogeneous mixing, the oil phase in the form of VCO is introduced into the mixture and stirred at the same speed and temperature. Then the mixture of ingredients is sonicated for 10 minutes. After the mixture of ingredients is sonicated, then aquadestilata is added drop by drop while stirring at a speed of 500 rpm. After that it is sonicated again for 10 minutes. Clove leaf ethanol extract of 500 mg is added to the carrier components (Tween 80 45 ml, propylene glycol 40 ml and VCO 5 ml) and then stirrer at a speed of 500 rpm. After that it is sonicated again for 10 minutes. The nanoemulsion characteristics were evaluated, specifically the determination of particle size employing a Particle Size Analyzer (PSA) and physical stability assessed via Zeta Potential measurements (Suhesti et al., 2025).

#### Acute Toxicity Test

As many as 25 rats were divided into 5 treatment groups and were calibrated for 14 days in the laboratory and fed and fed standard drinks. On day 0 all mice were fasted for 14-18 hours (constant drinking was given). All the rats that had been starved were weighed in

weight. Then the test preparation was administered orally in a single dose given within 24 hours. The control group was only given a nanoemulsion base, the Treatment I group (P1) was given a nanoemulsion test preparation of clove leaf extract with a dose of 5 mg/kg BB, Treatment II (P2) was given a nanoemulsion test preparation of clove leaf extract at a dose of 50 mg/kg BB, treatment III group (P3) was given a nanoemulsion test preparation of clove leaf extract at a dose of 300 mg/kg BB, Treatment IV group (P4) was given a nanoemulsion test preparation of clove leaf extract with a dose of 2000 mg/kg BB. After being treated, the feed can be given again after 3-4 hours and then observed for 14 days (BPOM, 2022).

*Weighing of Test Animal Bodies*

The weight of the test animal is carried out to observe the change in body weight in real time, then the test is an early indicator of the toxic effect of the given test sample and is the easiest to see. Test animals that experience toxicity generally lose weight due to decreased appetite (Khalishah et al., 2021). All male white rat test animals (*Rattus norvegicus*) weighed daily until day 14 after administration of clove leaf ethanol extract nanoemulsion preparation.

*Observation of Toxicity Symptoms*

After administration of the test preparation, the test animal is monitored for the first 30 minutes, then every 4 hours for the next 24 hours, then once a day for the next 14 days (BPOM, 2022). The toxic symptoms observed were pyloerxesis, ptosis, seizures, tremors, lacrimation, grooming, defecation and weakness.

*Determination of LD50*

The LD50 value is determined by the AOT425 StatPgm program. AOT425 StatPgm (Acute Oral

Toxicity Guideline 425 Statistical Program) is a software for calculating LD50 values (Kurniawan et al., 2022). The purpose of this procedure is to test the short-term toxicity of a chemical compound given to rodents. The information entered into the AOT425 StatPgm application is the dose and response of the test animal (dead/live) (Nasution et al., 2023). The procedure for calculating LD50 with AOT425 StatPgm takes place in stages (Ningrum, 2013).

*Data Analysis*

The data obtained in this study is primary data from the observation of test animals. The data obtained were in the form of quantitative and qualitative data. The quantitative data obtained were the number of dead test animals and their weight. LD50 data were taken from the number of dead and living rats in each treatment group. Then the LD50 value was tested with AOT 425 Statpgm limited software. The qualitative data obtained in the form of observation of the behavior of the test animals included changes in piloeraction activity, ptosis, seizures, tremors, lacrimation, grooming, defecation and weakness.

**Result and Discussion**

*Result*

*Phytochemical Screening*

The thick extract of clove leaves obtained was 98. The percentage of the yield is 20%. The results of phytochemical screening of ethanol extract of clove leaves can be seen in Table 1. Clove leaves (*Syzygium aromaticum L.*) contains seunder metabolites of flavonoids, saponins, steroids and triterpenoids.

**Table 1.** Phytochemical Test of Clove Leaf Ethanol Extract

| Chemical content       | Reagents                      | Observation Results  | Information |
|------------------------|-------------------------------|--|-------------|
| Alkaloid               | Dragendorff's reagent         | There is no orange red precipitate                             | -           |
| Flavonoid              | Concentrated HCL and Mg metal | A red purple color is formed                                   | +           |
| Saponin                | Shaken + HCl 2N               | Foam forms and remains for no less than 1 minute.              | +           |
| Tannin                 | FeCl3                         | No blue-black color is formed                                  | -           |
| Steroids/triterpenoids | H2SO4 + anhydrous acetic acid | Green color (steroid) and red color (triterpenoid) are formed. | +           |

**Table 2.** Clove Leaf Ethanol Extract Nanoemulsion Formula

| Material                   | Sum       | Uses              |
|----------------------------|-----------|-------------------|
| Clove leaf ethanol extract | 500 mg    | Active substances |
| VCO                        | 5 ml      | Oil phase         |
| Tween 80                   | 45 ml     | Surfaktan         |
| Propilenglikol             | 40 ml     | Ko-Surfaktan      |
| Aquadest                   | Ad 100 ml | Water phase       |

*Nanoemulsion Characterization*

The nanoemulsion showed average droplet size of 13.3 nm, consistent with optimal sonication formulations achieving 10-20 nm (Suhesti et al., 2025). Zeta potential value indicated good stability (Aini et al., 2022). Particle size reduction enhances bioavailability (Nugraha et al., 2023).

### Test Animal Weight Monitoring

Body weight of each group on day 1 before treatment and day 14 after treatment showed significant differences in each group marked by a P value of < 0.05. The results of weight observation can be seen in Table 3. Observation of Clinical Symptoms Clinical signs in mice

were observed in the first 30 minutes then 4 hours for the next 24 hours after administration of the test preparation, then once a day for 14 days. The results of the observation of clinical symptoms can be seen in Table 4. The clinical symptoms that occur are piloerection, grooming and weakness.

**Table 3.** Formula of Ethanol Extract Nanoemulsion of Clove Leaves

| Group              | Average Weight of Rats (grams) $\pm$ SD |                   | P     |
|--------------------|---|-------------------|-------|
|                    | Day-1                                   | Day-14            |       |
| Normal Controls    | 194.8 $\pm$ 7.463                       | 230.2 $\pm$ 6.380 | 0.000 |
| Dosage 5 mg/kgBB   | 195 $\pm$ 1.871                         | 206.2 $\pm$ 7.120 | 0.009 |
| Dosage 50 mg/kgBB  | 201.6 $\pm$ 3.362                       | 219.8 $\pm$ 8.526 | 0.006 |
| Dosage 300 mg/kgBB | 206.6 $\pm$ 2.074                       | 220 $\pm$ 4.637   | 0.001 |

### Discussion

Toxicity tests were carried out to determine the activity contained in the nanoemulsion of clove leaf ethanol extract. Nanoemulsions can better deliver drugs to smaller units in the body, overcome the resistance caused by the body's physiological barrier, can be targeted so as to reduce toxicity and improve the efficiency of drug distribution, Increased bioavailability of drugs with low absorption, reduced risk of side effects due to the use of drugs that irritate the gastrointestinal tract, accelerated drug dissolution time and increased drug dispersion (Magfirah et al., 2023).

This acute toxicity test uses male white rat test animals that have received permission from the Research Ethics Committee of Tadulako University with an ethical approval letter number 3047/UN28.10/KL/2024. Male white rats were selected for testing because male white rats are known to be good experimental model animals, easy to handle, and can be obtained in large numbers, with more stable research results because they are not affected by the menstrual cycle and pregnancy like female white rats. The Wistar strain was chosen because Wistar mice have a relatively fast metabolic capacity, so they are more sensitive when used in testing (Lahamendu et al., 2019).

The mice used for the experiment were acclimatized first for 14 days to adapt to the environment of the test site. The acclimatized rats were grouped into five groups of five mice each. Before treatment, the rats were fasted for 16 hours so that the rat's stomach was empty, which was expected not to affect the observation process of the acute toxicity test. After fasting, the animals are weighed and given a test preparation, the test preparation is administered in a single dose using a sonde. After treatment, the feed is re-fed after 3-4 hours.

Phytochemical test or phytochemical screening is a qualitative test of the content of secondary metabolite compounds using reagents. Compound identification is done visually by looking at the color change after the

reagent is added. The results of phytochemical screening tests show that clove leaf ethanol extract contains flavonoid compounds, saponins and steroids, this is in accordance with the literature stating that clove leaves contain saponins, phenols, terpenoids and flavonoids (Arni et al., 2023).

On the 1st day before administration and the 14th day after the administration of clove leaf ethanol extract nanoemulsion preparation, the results of the Anova test showed that the weight of the rats differed significantly between the normal control group, the dose of 5 mg/kg BB, the dose of 50 mg/kg BB, the dose of 300 mg/kg BB and the dose of 2000 mg/kg BB with a value of ( $P < 0.05$ ) so that it was continued with the Duncan test. The results of further tests by Duncan showed that the 5 mg/kg BB dose group was significantly different from the 50 mg/kg BB dose group, the 300 mg/kg BB dose and the normal control group, and then the 50 mg/kg BB dose group and the 300 mg/kg BB dose group were not significantly different, but significantly different from the 5 mg/kg BB dose and the normal control group.

The results of weight observation on the 1st day before administration and the 14th day after the administration of clove leaf ethanol extract nanoemulsion preparation in Table 3 of the T test showed a significant difference with a value of ( $P < 0.05$ ) in each treatment group. In the normal control group, there was an increase in weight by 18.74%, a dose of 5 mg/kg BB with an increase of 5.74%, a dose of 50 mg/kg BB with an increase of 9.02% and a dose of 300 mg/kg BB with an increase of 6.48%. This is in accordance with the literature that states that a good test animal is a test animal that has passed acclimatization and gained no more than 20% in weight and shows normal behavior (Nursafia, 2021). Weight changes are influenced by internal factors of genes which are the determining factors of traits inherited from the mother and hormones that will regulate all activities in the body. External factors such as food, activity, temperature and environment (Dewanti et al., 2023). In addition, weight

changes are also affected by the amount of feed intake (Harami et al., 2020). Similar findings on body weight monitoring during toxicity testing have been reported in the acute toxicity study of *Abelmoschus manihot*

ethanol leaf extract where no significant differences in body weight were observed during the 14-day observation period (Tuldjanah et al., 2023).

**Table 4.** Results of Observation of Clinical Symptoms in Normal Controls

| Clinical Symptoms | Percentage of Symptoms Clock to |         |         |          |          |          |        |
|-------------------|---------------------------------|---------|---------|----------|----------|----------|--------|
|                   | 30 minutes                      | 4 hours | 8 hours | 12 hours | 16 hours | 20 hours | 24 jam |
| Pyroerexy         | 100                             | 20      | 0       | 0        | 0        | 0        | 0      |
| Ptosis            | 0                               | 0       | 0       | 0        | 0        | 0        | 0      |
| Stiff             | 0                               | 0       | 0       | 0        | 0        | 0        | 0      |
| Tremor            | 0                               | 0       | 0       | 0        | 0        | 0        | 0      |
| Lacrificial       | 0                               | 0       | 0       | 0        | 0        | 0        | 0      |
| Grooming          | 100                             | 60      | 0       | 0        | 0        | 0        | 0      |
| Defecation        | 0                               | 0       | 0       | 0        | 0        | 0        | 0      |
| Salisation        | 0                               | 0       | 0       | 0        | 0        | 0        | 0      |
| Limp              | 100                             | 40      | 0       | 0        | 0        | 0        | 0      |

**Table 5.** Results of Observation of Clinical Symptoms at Dosage 5 mg/kg BW

| Clinical Symptoms | Percentage of Symptoms Clock to |         |         |          |          |          |          |
|-------------------|---------------------------------|---------|---------|----------|----------|----------|----------|
|                   | 30 minutes                      | 4 hours | 8 hours | 12 hours | 16 hours | 20 hours | 24 hours |
| Pyroerexy         | 80                              | 20      | 20      | 0        | 0        | 0        | 0        |
| Ptosis            | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Stiff             | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Tremor            | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Lacrificial       | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Grooming          | 100                             | 40      | 20      | 0        | 0        | 0        | 0        |
| Defecation        | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Salisation        | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Limp              | 100                             | 40      | 20      | 0        | 0        | 0        | 0        |

**Table 6.** Results of Observation of Clinical Symptoms at Dosage 50 mg/kg BW

| Clinical Symptoms | Percentage of Symptoms Clock to |         |         |          |          |          |          |
|-------------------|---------------------------------|---------|---------|----------|----------|----------|----------|
|                   | 30 minutes                      | 4 hours | 8 hours | 12 hours | 16 hours | 20 hours | 24 hours |
| Pyroerexy         | 100                             | 40      | 0       | 0        | 0        | 0        | 0        |
| Ptosis            | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Stiff             | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Tremor            | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Lacrificial       | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Grooming          | 100                             | 40      | 0       | 0        | 0        | 0        | 0        |
| Defecation        | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Salisation        | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Limp              | 100                             | 20      | 0       | 0        | 0        | 0        | 0        |

**Table 7.** Results of Observation of Clinical Symptoms at a Dosage of 300 mg/kg BW

| Clinical Symptoms | Percentage of Symptoms Clock to |         |         |          |          |          |          |
|-------------------|---------------------------------|---------|---------|----------|----------|----------|----------|
|                   | 30 minutes                      | 4 hours | 8 hours | 12 hours | 16 hours | 20 hours | 24 hours |
| Pyroerexy         | 100                             | 60      | 60      | 0        | 0        | 0        | 0        |
| Ptosis            | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Stiff             | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Tremor            | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Lacrificial       | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Grooming          | 80                              | 60      | 40      | 0        | 0        | 0        | 0        |
| Defecation        | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Salisation        | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Limp              | 100                             | 100     | 100     | 0        | 0        | 0        | 0        |

**Table 8.** Results of Observation of Clinical Symptoms at a Dosage of 2000 mg/kg BW

| Clinical Symptoms | Percentage of Symptoms Clock to |         |         |          |          |          |          |
|-------------------|---------------------------------|---------|---------|----------|----------|----------|----------|
|                   | 30 minutes                      | 4 hours | 8 hours | 12 hours | 16 hours | 20 hours | 24 hours |
| Pyroerexy         | 100                             | 40      | 40      | 0        | 0        | 0        | 0        |
| Ptosis            | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Stiff             | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Tremor            | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Lacrificial       | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Grooming          | 100                             | 40      | 20      | 20       | 0        | 0        | 0        |
| Defecation        | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Salisation        | 0                               | 0       | 0       | 0        | 0        | 0        | 0        |
| Limp              | 100                             | 60      | 40      | 20       | 0        | 0        | 0        |

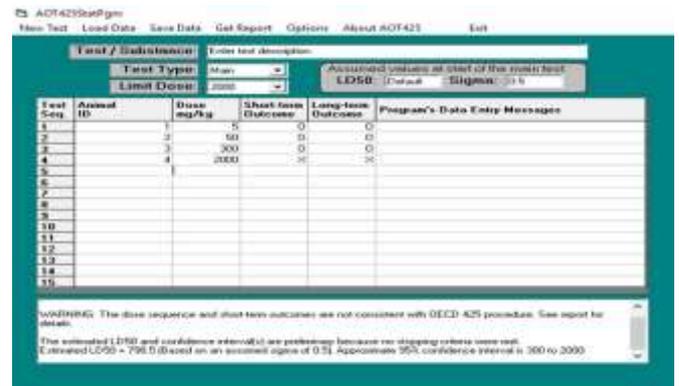
The results of observation of clinical symptoms in general in Table 4 showed that in the normal control group and the treatment group, there were no symptoms of ptosis, seizures, tremors, lacrimation, defecation and salivation. However, it shows symptoms of pyloerexy, grooming and weakness. Pyroereces that occur are caused by a sensitivity reaction to touch, spontaneous activity due to stimulation of the central nervous system. In each treatment group, there was a high piloereksi at the 30th minute and decreased at the 4th and 8th hours (Astuti, 2017). Grooming that occurs is caused by depression of the central nervous system (Dewanti et al., 2023). The high frequency of grooming at 30 minutes is considered an indicator of increased sensitivity to pain. In addition, grooming can be caused by the adaptation of rats to the test material that enters the body (Majid et al., 2023). The decrease in activity or weakness occurs due to a disorder of the nervous system that controls the muscles so that the rat is weak and there is a decrease in movement activity (Setiawan et al., 2023). Sub-chronic toxicity studies have shown that prolonged administration of plant extracts can affect vital organs including the kidneys (Nasir et al., 2024).

**Table 9.** Result of Observation of Death of Test Animals

| Dosage Groups   | Number of Rats | Number of Deaths |
|-----------------|----------------|------------------|
| Normal Controls | 5              | 0                |
| 5 mg/kg BB      | 5              | 0                |
| 50 mg/kg BB     | 5              | 0                |
| 300 mg/kg BB    | 5              | 0                |
| 2000 mg/kg BB   | 0              | 5                |

The results of observation of the death of test animals on the administration for normal control of nanoemulsion base and the administration of clove leaf ethanol extract nanoemulsion at doses of 5 mg/kg BB, 50 mg/kg BB and 300 mg/kg BB did not occur in test animals. Meanwhile, at a dose of 2000 mg/kg BB, death occurred in all test animals, indicating that the preparation of ethanol extract nanoemulsion of clove leaves was toxic at that dose, so that the LD50 obtained through the AOT425 StatPgm program was 798.5 which

was included in the category of moderate toxicity with an LD50 value range of >500-2000 mg/kg (BPOM, 2022).



**Figure 1.** LD50

**Conclusion**

Based on the results of the study, it can be concluded that the administration of nanoemulsion preparations of clove leaf ethanol extract at a dose of 5 mg/kgBB, a dose of 50 mg/kgBB, a dose of 300 mg/kgBB does not show toxic properties to male white rats, nanoemulsion of clove leaf ethanol extract at a dose of 2000 mg/kg BB can cause toxic effects on male white rats and the nanoemulsion of clove leaf ethanol extract has an LD50 value of 798.5 mg/kg BB, based on the GHS, it falls under Acute Toxicity (Oral) Category 4 (300–2000 mg/kg BW). Clove leaf ethanol extract nanoemulsion is considered safe for oral use at doses below 300 mg/kg BW and can be further developed into a standardized herbal medicine. Nanoformulation improves delivery of bioactive compounds like eugenol, warranting advanced studies.

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It is recommended to the next researcher to investigate the subchronic toxicity of the nanoemulsion preparation of clove leaf ethanol extract (*Syzygium aromaticum* L.)

**Author Contributions**

Conceptualization, M.T and R.Y ; Methodology, M.T and A.W; Formal analysis, M.T and P.N.L.L.; Investigation, M.T.;

Resources, R.Y.; Writing—preparation of original draft, P.N.L.L.; Writing—reviewing and editing, M.I.S.; Visualization, A.Y and M.I.S.; Supervision, J.T.; Project administration, M.T and P.N.L.L.; Obtaining funding, STIFA Pelita Mas Palu. All authors have read and approved the published version of the manuscript.

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### Conflicts of Interest

The authors declare no conflict of interest.

### References

- Aini, N. N., Wijayatri, R., & Pribadi, P. (2022). Nanoemulsion Characteristics of Ethanol Extract From Leave of Various Plants: A Literature Review. *Jurnal Farmasi Sains Dan Praktis*, 8(3), 215–226. <https://doi.org/10.31603/pharmacy.v8i3.4786>
- Arni, D. P., Idrus, I., & Nurtina, W. O. (2023). Formulasi Sediaan Salep Ekstrak Etanol (C<sub>2</sub>H<sub>5</sub>OH) Daun Cengkeh (*Syzygium aromaticum* L.) Sebagai Antibakteri. *Pelita Sains Kesehatan*, 3(3), 66–77. Retrieved from <https://ojs.pelitaibu.ac.id/index.php/jpasaik/article/view/65/48>
- Astuti, N. (2017). Uji Toksisitas Akut Ekstrak Etanol Daun Pletekan (*Ruellia tuberosa* L.) Pada Mencit Betina (*Mus musculus*) Galur BALB/c. Universitas Setia Budi Surakarta.
- Batiha, G. E. S., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). *Syzygium aromaticum* l. (myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, 10(2). <https://doi.org/10.3390/biom10020202>
- BPOM. (2022). Peraturan Badan Pengawas Obat Dan Makanan Nomor 10 Tahun 2022 Tentang Pedoman Uji Toksisitas Pratinik Secara In Vivo. Badan Pengawas Obat dan Makanan Republik Indonesia.
- Chioman, L., Sebayang, D. P. A., Chioman, V., & Suhartomi, S. (2024). Anti-inflammatory Activity of Lemon Pepper Nanoemulsion on Carrageenan-Induced Male Wistar Rat. *Jurnal Penelitian Pendidikan IPA*, 9(SpecialIssue), 1428–1434. <https://doi.org/10.29303/jppipa.v9iSpecialIssue.6292>
- Dewanti, S. P., Iswandi, I., & Sasangka, N. A. D. (2023). Uji Toksisitas Akut Ekstrak Etanol Daun Salam (*Syzygium polyanthum*) pada Mencit Jantan (*Mus musculus*). *Borneo Journal of Pharmascientech*, 7(2), 46–51. <https://doi.org/10.51817/bjp.v7i2.470>
- Dewi, S., Redhita, L. A., Beandrade, V. P., Fitriani, N., & Handayani, R. (2022). Formulasi nanoemulsi ekstrak daun kemangi dengan Tween 80. *Jurnal Mitra Kesehatan*, 4(2), 80–91. <https://doi.org/10.29303/jmk.v4i2.134>
- Harami, G. M., Kovács, Z. J., Pancsa, R., Pálinkás, J., Baráth, V., Tárnok, K., Málnási-Csizmadia, A., & Kovács, M. (2020). Phase separation by ssDNA binding protein controlled via protein-protein and protein-DNA interactions. *Proceedings of the National Academy of Sciences of the United States of America*, 117(42), 26206–26217. <https://doi.org/10.1073/pnas.2000761117>
- Kamaruddin, S. Z. A. (2021). Uji Toksisitas Akut Minyak Cengkeh (*Oleum Caryophylli*) Terhadap Struktur Jaringan Hati Tikus (*Rattus Norvegicus*) Acute Toxicity Test Of Clove Oil (*Oleum Caryophylli*) On The Structure Of Rat (*Rattus Norvegicus*) Liver Tissue. Universitas Hasanuddin.
- Khalishah, H., Kurniawan, H., Nugraha, F., Nurbaeti, S. N., & Fajriaty, I. (2021). Pengaruh Pemberian Serbuk Cangkang Telur Terhadap Bobot Badan dan Indeks Organ Tikus Putih (*Rattus norvegicus* L.) Galur Wistar dalam 28 Hari. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*, 5(1), 1–6. Retrieved from <https://jurnal.untan.ac.id/index.php/jmfarmasi/article/view/49023>
- Kuncoro, B., Putri, L. A., & Supriyanta, J. (2023). Formulasi Dan Evaluasi Fisik Sediaan Ovula Nanoemulsi Minyak Atsiri Daun Cengkeh (*Syzygium Aromaticum* L.). *Jurnal Farmagazine*, 10(2), 33–42. <https://doi.org/10.47653/farm.v10i2.712>
- Kurniawan, A., & Norwalidaini, F. (2022). Uji toksisitas akut ekstrak etanol 70% daun kersen (*Muntingia calabura* L.) dengan metode OECD 425. Universitas Bangka Belitung.
- Lahamendu, B., Bodhi, W., & Siampa, J. P. (2019). Uji Efek Analgetik Ekstrak Etanol Rimpang Jahe Putih (*Zingiber officinale* Rosc.var. *Amarum*) pada Tikus Putih Jantan Galur Wistar (*Rattus norvegicus*). *Pharmacon*, 8(4), 927. <https://doi.org/10.35799/pha.8.2019.29372>
- Lumingkewas, A. M. W., & Unitley, A. J. A. (2023). Identification of Phytochemical Compounds Antioxidant Leaf Extract and Clove Flower Extract (*Syzygium aromaticum*). *Jurnal Penelitian Pendidikan IPA*, 9(10), 8533–8538. <https://doi.org/10.29303/jppipa.v9i10.4927>
- Magfirah, M., Utami, I. K., Nurllia K.D, I. D. A., & Dewi, N. P. (2023). Optimization and Validation Test of Self Nano-emulsifying Drug Delivery System Capsule of Ethanol Extract Parang Romang Leaves. *Ad-Dawaa' Journal of Pharmaceutical Sciences*, 6(1), 67–76.

- <https://doi.org/10.24252/djps.v6i1.38469>
- Mahiya, Ratnasari, D., & Utami, M. R. (2022). Optimasi Formula SNEDDS (Self-Nanoemulsifying Drug Delivery System) Ekstrak Daun Kejibeling Menggunakan Metode SLD (Simplex Lattice Design). *Jurnal Ilmiah Wahana Pendidikan*, 8(11), 124–129. <https://doi.org/10.5281/zenodo.6830330>
- Majid, G. A., Keswara, Y. D., & Ansory, H. M. (2023). Pengujian Toksisitas Akut Minyak Atsiri Biji Pala pada Mencit Jantan (*Mus musculus*). *Jurnal Farmasi Indonesia*, 20(2), 149–163. <https://doi.org/10.31001/jfi.v20i2.2263>
- Maulidza, C. P., Halim, B., Chiuman, L., Nasution, A. R., & Theresia, Y. (2025). The Effect of Andaliman Fruit Extract (*Zanthoxylum acanthopodium* DC) on  $\alpha$ -Synuclein Levels in Rotenon-Induced Wistar Rats. *Jurnal Penelitian Pendidikan IPA*, 11(2), 516–524. <https://doi.org/10.29303/jppipa.v11i2.10362>
- Merlani, V., & Febrina, D. (2024). Studi Toksisitas Akut Ekstrak Etanol Bunga Telang (*Clitoria ternatea* L.) pada Tikus Putih. *Jurnal Ilmu Farmasi Terapan Dan Kesehatan*, 2(1), 17–27. <https://epik.ikifa.ac.id/journals/article/view/94>
- Mustapa, M. A. (2018). Uji Toksisitas Akut Yang Diukur Dengan Penentuan Ld50 Ekstrak Etanol Bunga Cengkeh (*Syzygium Aromaticum* L.) Terhadap Mencit (*Mus Musculus*) Menggunakan Metode Thompson-Weil. *Frontiers: Jurnal Sains Dan Teknologi*, 1, 105–117. <https://doi.org/10.36412/frontiers/001035e1/april201801.10>
- Nasiro, S., Anjulita, R., Setiawan, E., Afriyandi, A., & Situmeang, B. (2023). Potensi Nanoemulsi Ekstrak Daun Pirdot (*Saurauia vulkani*) dalam Meningkatkan Aktivasi Enzim Alpha-Amilase sebagai Alternatif Terapi Diabetes Mellitus. *Jurnal Beta Kimia*, 3(2), 67–74. <https://doi.org/10.35508/jbk.v3i2.15164>
- Nasution, A. N., Fitriasia, R., Tio, S., Girsang, E., & Mutia, M. S. (2023). Formulation and Characterization of *Amomum Compactum* Sol. Ex Maton Nano Emulsion and Its Antibacterial and Wound Healing Activity. *Jurnal Penelitian Pendidikan IPA*, 9(12), 10656–10662. <https://doi.org/10.29303/jppipa.v9i12.5773>
- Ningrum, S. R. W. (2013). *Validasi Uji Toksisitas Akut Metode Oecd 425 Pada Mencit Betina Menggunakan Tembaga (Ii) Sulfat Pentahidrat*. Universitas Indonesia Press.
- Nugraha, A., & Nurhayati, N. (2023). Formulasi nanoemulgel ekstrak buah lada hitam. *Farmasetika*, 8(1), 50–60. <https://doi.org/10.24198/jfs.v8i1.40678>
- Nursafia, Y. (2021). Uji Toksisitas Akut Dari Ekstrak Etanol Daun Kayu Manis (*Cinnamomum burmanni*) Terhadap Mencit Putih (*Mus musculus* L.) Betina. *Frontiers in Neuroscience*, 14(1), 1–13. <https://doi.org/10.36387/jiis.v6i2.720>
- Putri, N. N., & Sari, D. P. (2024). *Optimasi konsentrasi Tween 80 dan etanol 96% dalam nanoemulsi ekstrak etanol daun sukun*. Universitas Sriwijaya.
- Qoriasmadillah, W., Haqiqi, N., Iman, Z., Setyaningrum, T. W., Candri, D. A., Frediansyah, A., & Prasedya, E. S. (2024). Kandungan Ekstrak Daun Banten (*Lannea coromandelica* (Houtt.) Merr.) Obat Tradisional Lombok Sebagai Antipiretik Pada Mencit (*Mus musculus*). *Jurnal Penelitian Pendidikan IPA*, 10(4), 1855–1862. <https://doi.org/10.29303/jppipa.v10i4.6756>
- Ramadhani, M. (2022). (*Syzygium aromaticum* L) Terhadap Aktivitas Antioksidan Nanopartikel Zein-Minyak Atsiri. Universitas Andalas.
- Sandhiutami, N. M. D., Dewi, R. S., Khairani, S., & Widyadari, S. A. M. (2022). Evaluasi Keamanan dari Pengembangan Formula Nanopartikel Kurkumin pada Mencit dan Potensi Antioksidan In-Vitro/Evaluasi Keamanan dari Pengembangan Formula Nanopartikel Kurkumin pada Mencit dan Potensi Antioksidan In-Vitro. *Jurnal Ilmu Kefarmasian Indonesia*, 20(1), 63. <https://doi.org/10.35814/jifi.v20i1.1187>
- Sari, N. M., & Kurniawan, R. (2024). Uji toksisitas akut ekstrak etanol 70% kulit batang tandui (*Mangifera rufocostata*). Universitas Bangka Belitung.
- Setiawan, Poernomo, H., & Pramana, R. (2023). Acute Toxicity Test Of Ambon Banana Tree Sap Extract On Mice (*Mus Muculus* L.). *Interdental Jurnal Kedokteran Gigi (IJKG)*, 19(2), 170–176. <https://doi.org/10.46862/interdental.v19i2.7480>
- Suhesti, T. S., & Muslimah, A. (2025). Nanoemulsion of Nagasari (*Mesua ferrea* L.) Leaf and Its Activity Against *Staphylococcus aureus*. *Indonesian Journal of Pharmaceutical Science and Technology*, 12(2), 137–143. <https://doi.org/10.24198/ijpst.v12i2.49866>
- Tuldjanah, M., Yanuarty, R., & Wulandari, A. (2023). Evaluasi Aktivitas Afrodisiak Etil Fraksi Asetat Daun Cengkih (*Syzygium aromaticum* L.) Pada Tikus Putih Jantan (*Rattus norvegicus*). *Medical Sains: Jurnal Ilmiah Kefarmasian*, 1569–1578. Retrieved from <https://shorturl.at/vOTbh>
- Yeni, L. F., Nurfatehah, Erma, & Sari, E. E. (2025). Antibacterial Activity of Indonesian Medicinal Plant Extracts *Tinospora crispa*, *Averrhoa bilimbi* and *Syzygium polyanthum* against *Shigella sonnei*. *Jurnal Penelitian Pendidikan IPA*, 11(4), 355–363. <https://doi.org/10.29303/jppipa.v11i4.9378>