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ACUTE TOXICITY TEST OF ROBUSTA COFFEE LEAVES EXTRACT USING BRINE SHRIMP LETHALITY TEST (BSLT)

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ABSTRACT

Robusta coffee leaves (*Coffea canephora*) are known to contain secondary metabolites such as flavonoids, alkaloids, tannins, phenolics, and steroids, which have the potential to exhibit pharmacological bioactivity. However, the toxicity profile of these leaves has not been widely reported. This study aims to determine the acute toxicity of the ethanol extract of robusta coffee leaves using *the Brine Shrimp Lethality Test* (BSLT) method. Extraction was carried out through the maceration method using 96% ethanol, followed by ethanol-free tests and phytochemical screening. The BSLT was performed on *Artemia salina L* larvae. with various concentrations of extracts, and a *Lethal Concentration value of 50%* (LC_{50}) was calculated based on larval mortality after 24 hours. The results showed that the ethanol extract of robusta coffee leaves contained flavonoids, alkaloids, tannins, phenolics, and steroids, with an LC_{50} value of 139.378 ppm, which was classified as moderately toxic. These findings suggest that the ethanol extract of robusta coffee leaves has the potential for acute toxicity according to the BSLT method and can be used as a basis for further research on the exploration of cytotoxic activity.

Keywords: *Coffea canephora*, BSLT, *Artemia salina* L., acute toxicity, LC_{50}

INTRODUCTION

Robusta coffee (*Coffea canephora*) is a coffee variety widely cultivated in Indonesia and is considered a major agricultural commodity (Hidayat *et al.*, 2024). Robusta coffee is known for its higher caffeine content than Arabica coffee and offers potential health benefits, such as antioxidant and antibacterial effects (Noviyandri *et al.*, 2020; Hosted *et al.*, 2022). In coffee plants, it is not only limited to the seeds, but can also be used as a natural drink from the leaves that is useful for health and as a raw material for traditional medicine.

Coffee leaves are a part of the coffee plant that is considered waste and has not been widely used to the fullest. Robusta coffee leaves contain several compounds that are secondary metabolites, including flavonoids, steroids, alkaloids, saponins, phenolics, and steroids (Damaiyanti *et al.*, 2023). According to Less & Kamengon (2021), the results of phytochemical screening of robusta coffee leaves were positive for alkaloids, flavonoids, terpenoids, and phenolics, which provide antioxidant activity with an IC50 of 56,337 ppm. In addition, larvicide tests on Aedes aegypti larvae revealed that the N-hexane extract from robusta coffee leaves was effective in eradicating larvae (Marcellia *et al.*, 2021). Shiyan *et al.* (2017) reported that the ethanolic extract of robusta coffee leaves has antidiabetic activity, with the largest percentage of decrease in blood glucose levels shown at a dose of 236 mg/kgBB with an ED50 value of 126.90 mg/kg BB. According to some of the studies above, it has been proven that robusta coffee leaves function as antioxidants, antibacterial, antidiabetic, and antilarvicide mosquitoes.

Various groups of toxic compounds derived from plants, such as coffee, have become an important field in health and environmental studies. As previously studied by Fitri *et al.* (2021) showed that ethanol extract from the fruit skin of robusta coffee has harmful effects on shrimp larvae.

Despite these findings, the toxicological profile of robusta coffee leaf extract remains largely unexplored. The results of several previous studies on Robusta coffee, fruit peels, and leaves provide preliminary information for further research using toxicity tests to search for natural products with potential antineoplastic properties. As an initial test to determine the toxicity properties of robusta coffee leaf extract, a toxicity test was carried out using the *Brine Shrimp Lethality Test* (BSLT), which is known as an efficient approach for evaluating the toxicity of natural materials, with the use of larvae *Artemia salina* as a model organism in testing (Pohan, et al., 2023). Shrimp larval mortality is used as a parameter to indicate the presence of cytotoxic plant active substances if the LC50 Price is <1000 µg/ml (Meyer *et al.*, 1982).

This study seeks to complement the existing information gap by using the BSLT approach to evaluate the toxicity of the robusta coffee leaf extract. This study aimed to use *Artemia salina* larvae to obtain data on the possible toxicity of robusta coffee leaf extract. This study will test the effect of extract concentration on larval mortality rates to determine the LC50 value, which indicates the threshold of extract toxicity. Referring to background exposure, the researcher was encouraged to explore the potential of robusta coffee leaf extract in *Artemia salina* larvae using the BSLT method. The goal of this study was to determine whether robusta coffee leaf extract has a toxic effect on shrimp larvae (*Artemia salina* L.). =

RESEARCH METHODS

Tools and materials

Tool

Digital analytical balance, blender, 40 mesh sieve, maceration vessel, measuring cup (*Pyrex*), tray, stirring rod, *aluminum foil*, filter paper, glass funnel, *beaker glass* (*Pyrex*), scissors, *rotary evaporator*, refrigerator, micro pipette, decicorer, thermometer, 40-60 watt incandescent/fluorescent lamp, aerator, Shrimp larval hatching set (Jebo), and Vial (Duran).

Material

Fresh robusta coffee leaves, *Artemia salina* L., ethanol 96%, aquades, Mayer's reagents, Dragendorff's reagents, seawater, concentrated HCl, Mg metals, HCl 2N, chloroform, ammonia, aquades, FeCl3 5%, KOH, FeCl3 1%, *Liberman Bouchardat's reagents*, and *Tween* 80.

Research Internships

Sample Determination

The robusta coffee leaf samples were determined at the botanical laboratory of the University of Lampung, Faculty of Mathematics and Natural Sciences, by matching the morphological characteristics found in the robusta coffee plant (*Coffea canephora*).

Sample Preparation

Robusta coffee leaves were sourced from plantations in Kota Besi Village, Batu Brak District, West Lampung, Lampung Province. Leaves that were fresh, green, and in good condition were selected. After harvesting, the leaves were sorted based on these criteria and washed under running water to remove any surface contaminants. The cleaned leaves were then cut into smaller pieces and dried under sunlight for 5–6 days by covering them with a black cloth. Once fully dried, the leaves were ground into a coarse powder, sieved, and stored in an airtight container.

Robusta Coffee Leaf Extraction

This study used a modified maceration method based on that described by Nuralifah *et al.* (2018). Robusta coffee leaf powder (500 g) was soaked in 96% ethanol at a sample-to-solvent ratio of 1:10, requiring 5000 mL of ethanol. The simplicia was first soaked in 3 liters of 96% ethanol for 24 hours, protected from light, stirred occasionally, and then filtered to obtain the first filtrate. The remaining residue is added with 2 liters of 96% ethanol and remacerated for

48 hours. All collected extracts were concentrated using a Rotary Vacuum Evaporator at 40–50℃ until a thick extract was obtained.

Ethanol-Free Test

The ethanol-free test of Robusta Coffee leaf extract was carried out with the addition of 2 mL of acetic acid (CH3COOH) and 2 mL of concentrated sulfuric acid (H2SO4) to a number of test solutions. After the mixture was homogenized, it was heated using a Bunsen burner. If the test results do not smell of ester, then the positive extract is ethanol-free (Klau *et al*., 2021).

Phytochemical Screening

Flavonoid Test

Flavonoid tests can be performed qualitatively using the Wilstatter technique. This requires a measurement of 0.5 grams of extract, followed by the addition of 2-4 drops of concentrated HCl or 2-3 small fragments of magnesium metal. The appearance of an orange-red color accompanied by light brown deposits indicates a positive response (Sawiji *et al*., 2022).

Alkaloid Test

A sample of 1 gram was added to 6 mL of hydrochloric acid 2N and 9 ml of aquadest heated over a water bath for 2 minutes, cooled, and filtered. The filtrate was used for alkaloid testing. 3 test tubes were taken, and 2 ml of the filtrate was inserted into each test tube. The first tube was added 5 drops of Mayer's reagent, the second tube was added 5 drops of Dragendorff reagent, and the third tube was added 5 drops of Bouchardat (Sulistyarini *et al*., 2020).

Tannin Test

The sample (0.5 g) was poured into 5 mL of hot water and filtered, and the resulting solution was placed into a tube-shaped reaction container. Next, a few drops of 1% FeCl were added. The appearance of a greenish-black color indicates the presence of tannins (Novi *et al*. 2024).

Saponin Test

A sample (0.5 g) was placed in 5 mL of hot water and filtered. The filtrate was reacted until foam was formed by dissolving the concentrated extract in hot distilled water, followed by shaking and adding HCl 2N. The presence of stable foam indicates a positive result for saponins (Novi *et al*., 2024).

Phenolic Tests

A few drops of the extract were added to a test tube, followed by the addition of a number of drops of 1% $FeCl_3$ solution. Positive indications are shown by a color change from purple to dark blue (Djohari *et al*., 2021).

Steroid Tests

The extract was dripped with 1-2 drops of the Liebermann–Burchard reactant, which consisted of 2 mL of concentrated H2SO4 and 2 mL of anhydrous acetate. Positive results were characterized by the presence of blue or green color (Djohari *et al*., 2021).

Toxicity Test Using Brine Shrimp Lethality Test (BSLT) Method

1. Preparation of Artemia salina larvae

Shrimp larvae were prepared by hatching them in light or dark conditions. Low-light zones serve as egg hatcheries and aerator installations, whereas brightly lit areas are equipped with bulb lights that provide light for the shrimp egg hatching process. A perforated barrier was inserted into the container to facilitate the natural migration of hatching larvae towards the light. The container contained one liter of seawater. In a low-light area, a teaspoon of eggs that had previously been washed and soaked in distilled water for one hour was carefully placed. Subsequently, the part was covered with aluminum foil to minimize light exposure. The brightly lit area was equipped with a 40-watt fluorescent lamp to maintain the stability of the

incubation temperature in the range of 25–30°C. The eggs were left submerged for 48 hours until the hatching process occurred, which generally lasted 18–48 hours, causing the larvae to instinctively migrate to a well-lit area, thus detaching from the eggshell. The actively swimming larvae were collected using a pipette and placed in a bottle filled with seawater to be used as test animals in this study (Lestari *et al*., 2019; Saragih *et al*., 2022).

2. Test Solution Manufacturing

Robusta coffee leaf ethanol extract (0.2 g) was placed in a 100 mL measuring flask, and seawater was added to dissolve the extract. If the sample is not dissolved, Tween 80 was added. Seawater was added to the pumpkin until the mark was reached, resulting in an ethanol extract with a concentration of 2000 ppm. Furthermore, samples with concentrations of 100, 250, 500, 750, and 1000 ppm were diluted, and negative controls were prepared, such as test samples without the addition of extracts.

3. Toxicity Test

The acute toxicity test was performed using the Brine Shrimp Lethality Test (BSLT) method with shrimp larvae that were 48 hours old. Each test concentration was prepared in a 2000 µl, with three replicates to improve the accuracy of the results. To each vial, 10 mL of sample solution was added, followed by the addition of 10 larvae. As a negative control (blank), the same procedure was applied in the absence of the addition of extracts. The entire vial was placed under 5 watt TL lamp to maintain optimal environmental conditions. Larval mortality was observed within 24 hours, and the number of larvae that did not survive was calculated manually. Larvae that were categorized as dead were those that did not show a movement response for a few seconds when observed (Lestari *et al*., 2019).

Data Analysis

The acute toxicity test was analyzed using the probit method by calculating the percentage of Artemia salina larval mortality using the following formula:

$$\%\text{Mortality} = \frac{Number\ of\ dead\ larvae}{Total\ number\ of\ initial\ larvae} \times 100\%$$

If a dead larva was found in the control group, the percentage of mortality was analyzed using the Abbott correction formula to obtain more accurate results (Meyer *et al*., 1982).

$$\%\ \text{Death} = \frac{Number\ of\ dead\ larvae\ -\ Dead\ larvae\ at\ control}{\text{Number of initial larvae.}} \times 100\%$$

The data analysis is displayed through tables and curves. Linear regression equations were created using Microsoft Office Excel to correlate the probit value and the concentration log, the LC50 value, which represents the concentration where there is a dependent variable (x), while the probit analysis data are considered independent variables (y). The hereditary regression equation was used to ascertain the concentration that caused 50% of the larvae to die.

Regression equation:

$$y = ax + b$$

Information:

A: Slope Regression

B: Intercept

x: Concentration log

y : Probit value y= 5.00 (50% death)

RESULTS AND DISCUSSION

In this study, we tested the toxicity of Robusta Coffee leaf extract (*Coffea canephora*) on shrimp larvae (*Artemia salina* L.) using the BSLT method. The Robusta Coffee leaves used in this study were obtained from coffee plantations in Kota Besi Village, Batu Brak District, West Lampung, Lampung Province. The Robusta Coffee leaves used are leaves that are still in good condition, undamaged, fresh, and green in color according to the criteria set by the researcher.

Before the research was conducted, a determination process was carried out to identify the specific names and types of plants. The goal is to ensure that the plants used are suitable for the desired species of interest. Robusta coffee plants were identified at the Botanical Laboratory of FMIPA, University of Lampung, with reference to the classification system described by Cronquist (1981). The results showed that the plant was robusta coffee, with the scientific name *of Coffea canephora*.

Before the extraction process of Robusta Coffee leaves, the leaves were first used as a simplia by means of robusta coffee leaves that had been obtained to be weighed with a fresh weight of 2.5 kg and then washed first with running water to remove impurities attached to the surface of the leaves. The leaves were then cut into small pieces to expand the surface area of the raw material and accelerate the drying process. After being cut into small pieces, they were dried. The purpose of drying is to reduce the moisture content and stop enzymatic reactions to obtain simplicia that are not easily damaged, so that they can be stored for a longer period of time (Apriani *et al*., 2022). The drying process was carried out by drying in the sun for 5–6 days and covering with a black cloth so that the sample was not exposed to direct sunlight and the active ingredients were not damaged. Damage to secondary metabolite compounds is caused by gamma rays, UV-B rays, and UV-C rays; therefore, the drying process is an important stage in the manufacture of simplicia, because it can affect the quality and content of active substances in simplicia (Novi, Lestari and Puspitasari, 2024). After reaching a dry condition, the leaves were then mashed until they obtained a coarse powder texture and then filtered using a sieve so that in the maceration process, the solvent could penetrate the cell wall that binds the active compounds contained in Robusta Coffee leaves. The dried powder of Robusta Coffee leaves was weighed as needed in this study using up to 500 g of powder that was ready to be extracted.

The extraction method used in this study was maceration. The principle of maceration is that chemical compounds with the same properties as solvents are attracted to and dissolved in the solvent, allowing for the separation of certain chemical compounds (Chusniasih and Tutik, 2020). The maceration method was chosen because it does not involve heating, which prevents the decomposition or damage of thermolabile chemical compounds. The advantage of this method is that the way of working and the equipment used are simple and easy to implement (Najib, 2018). Maceration was performed using 96% ethanol. Ethanol was chosen as the solvent because it is universal, polar, and easy to obtain. Ethanol (96%) was also chosen because it is selective, non-toxic, has good absorption, and high filtration ability, and can be used to detect non-polar, semi-polar, and polar compounds. Ethanol solvents are 96% easier to penetrate the sample cell wall than lower concentrations, resulting in a concentrated extract (Wendersteyt *et al*., 2021).

The maceration process was carried out by weighing 500 g of simplicia, then soaking it using 96% ethanol solvent as much as 3 liters for 24 hours, protected from light, and stirred occasionally, after which the pulp and filtrate were separated using filter paper. The refining process is then repeated with the same type of solvent and a solvent volume of 2 liters for 2 times 24 h. This is called remaceration, which aims to attract the content of compounds that are still left behind at the time of the first maceration. Subsequently, all the mafibers were collected, and the solvent was evaporated using *a Rotary Vacuum Evaporator* at 40°C.

Extraction was concentrated at a temperature of ±40°C because it is considered safe and does not damage the metabolite compounds contained in the sample (Apriani *et al*., 2022). Evaporation was performed to separate the solvent from the extract to obtain a thick extract. Robusta Coffee leaf extract obtained after evaporation was in the form of a dark green thick extract of 46.48 grams from 500 grams of dried Robusta Coffee leaves with a yield of 9.29%.

This value is different from the results obtained by Rosalia *et al.* (2021), who reported a 10.80% yield from the extraction of robusta coffee leaves using percolation with a 96% ethanol solvent. The difference in yield values can be influenced by several factors, such as differences in extraction methods and material-solvent comparisons. Rosidah *et al*. (2017) also confirmed that the choice of extraction method affects the efficiency of the acquisition of phenolic compounds and other secondary metabolites, where percolation often results in higher yields than maceration. The substance-solvent ratio and the amount of extracts involved in the transfer determine the degree of concentration difference, which is very important in the diffusion process that affects the content of the compound.

Table I. Yield Results of Robusta Coffee Leaf Extract (*Coffea canephora*)

Sample	Wet Weight (Grams)	Solvent (L)	Simplisia Weight (grams)	Extract Weight (grams)	Yields (%)
Robusta Coffee Leaves	2500	5	500	46,48	9,29%

The condensed extracts obtained are subjected to ethanol-free testing The purpose of this test is to ensure that the extract is pure extract and there is no ethanol content in it before being used for the toxicity test used does not produce biased data (Prasticha and Surahmaida, 2024). If ethanol residues are still present in the sample, larval death may be caused by ethanol and not by the bioactive compounds in the extract. The test results show that the test results of the condensed extract of Robusta Coffee leaves does not smell of esters, and it can be said that the condensed extract of Robusta Coffee leaves is free of ethanol.

The next step was to conduct a phytochemical screening test on the extract results. This phytochemical screening aims to determine the secondary metabolite compounds present in the plant. In this study, the phytochemical screening test of ethanol extract of Robusta Coffee leaves showed positive sample results containing flavonoids, alkaloids, tannins, phenolics, and steroids, but negative results for saponins, characterized by no stable foam formation after shaking and the addition of HCl 2N. These results differ from those of Rosalia *et al.* (2021), who reported that the ethanol extract of robusta coffee leaves showed positive results for saponins, characterized by the formation of a 1-10 cm high foam that was stable for 15 minutes. This difference in results can be caused by differences in the ratio of materials to solvents during extraction, which affects the concentration of secondary metabolites extracted, differences in saponin tests performed by adding HCl 2N after shaking, and differences in extraction methods.

The flavonoid groups were identified by adding concentrated Mg and HCl metals to the sample. The test results were positive, with a color change to reddish-yellow. The reaction results from the reduction of the benzopyrone nucleus in the flavonoid structure, leading to an orange or red color change (Novi *et al*., 2023).

Alkaloid groups were identified using three reagents: *Mayer, Dragendorff,* and *Bouchardat*. The presence of alkaloids was determined based on sediment formation. A positive reaction with Mayer's reagent is characterized by the appearance of yellowish-white deposits, which occur due to the interaction between alkaloids and tetraiodomercurate (II) ions, resulting in precipitated complex compounds. The presence of alkaloids was confirmed by the formation of orange-colored deposits, which are caused by the interaction between the compound and the tetraiodobismutate (III) ions (Sulistyarini, Sari and Wicaksono, 2020). Positive results with Bouchardat's reagents are characterized by the formation of dark yellow to brownish deposits. This occurs through the coordination of covalent bonds between K^+ ions and alkaloids, resulting in the formation of a potassium–alkaloid precipitating complex. Potassium iodide and iodine are involved in this reaction (Sulistyarini, Sari and Wicaksono, 2020).

Tannin testing on Robusta Coffee leaf extract was carried out with the addition of FeCl3, and the test results showed that positive results were characterized by a change in the color of the solution to blackish-green after the addition of FeCl3. Tannin compounds are polar due to the presence of OH groups; therefore, when a sample is added to 10% FeCl3, there will be a color change, such as dark blue or blackish-green, which indicates the presence of tannin compounds (Sulistyarini *et al*., 2020).

Testing of phenol compounds on Robusta Coffee leaf extract obtained positive results, where there was a change in the color of the sample to blackish-green after being reacted with FeCl3. This is because phenolic compounds react with 1% FeCl3 and form deep red, purple, blue, or black colors because FeCl3 reacts with aromatic –OH groups (Nurcholis *et al*., 2022).

Steroid testing of the sample showed a green color in the ethanol extract of Robusta coffee leaves. This is because steroids that react with anhydrous acetic acid and a drop of concentrated sulfuric acid produce a green or blue color. The reaction that occurs between steroids and anhydrous acetic acid is the acetylation of the –OH group on steroids (Sulistyarini *et al*., 2020).

Table II. Phytochemical Screening Results of Robusta Coffee Leaf Extract

Sample	Identification	Observation Results	Information
Robusta Coffee Leaf Extract	Flavonoids	Reddish yellow (*orange*)	+
	Alkaloids		
	- Mayer	Yellow deposits	+
	- Dragendorf	Orange deposits	+
	- Bouchardat	Blackish deposits	+
	Tannins	Blackish Green	+
	Saponins	No foam	-
	Phenol	Brownish yellow	+
	Steroids	Changing color to Green	+

Information:
(+) : contains a group of secondary metabolite compounds tested
(-) : does not contain the secondary metabolite compound group tested

Toxicity testing using the Brine Shrimp Lethality Test (BSLT) on shrimp larvae (Artemia salina L.) is widely used to evaluate the cytotoxic effects of bioactive chemicals. This is the initial toxicity screening for the plant extracts (Sarah *et al*., 2017). This test is also used as a bioassay guide to detect cytotoxic and antitumor activities. The advantages of this method are that it is fast (results within 24 hours), cheap, simple (without aseptic techniques), uses easily obtained organisms, does not require special equipment, and requires few test samples. The obtained active compounds can be further tested for their specific pharmacological activities (Rani *et al*., 2022).

Shrimp larvae (Artemia salina L.) were used in the toxicity test because they have high sensitivity to chemical compounds from both heavy metals and medicinal plants (Konan *et al*., 2022). In addition, the use of large amounts of Artemia salina L. (3 replication) allows for robust statistical analysis of test samples (Sarah *et al*., 2017).

Hatching larval eggs (Artemia salina L.) were used as much as 10 mg hatched in 250 mL of seawater and left for 2 x 24 hours. The use of larvae that are 48 hours old because at this age, they are in a sensitive state (Saragih *et al*., 2022), the larvae are actively mobile, and they have a high rate of mitotic division. This high mitotic activity makes Artemia salina L. larvae similar to cancer cells, making them suitable for evaluating the effects of chemical compounds on cancer cell growth (Andini *et al*., 2021). In addition, 48-hour-old A. salina larvae have complete organs and a high level of sensitivity to chemical substances, allowing for the accurate detection of cytotoxic effects (Ntungwe *et al*., 2020).

After the Artemia salina eggs hatched, five test concentrations were prepared: 1000, 750, 500, 250, and 100 ppm. The test solution was prepared from a stock solution of 2000 ppm, and each extract was placed into a 10 mL measuring flask. The sample was then dissolved in

seawater using Tween 80. Plant extracts typically contain a variety of secondary metabolites with different degrees of polarity, such as relatively polar alkaloids and flavonoids, non-polar terpenes, and semi-polar steroids. While seawater is polar. Owing to these differences, the sample did not completely dissolve using only seawater. Tween 80 1% works as a surfactant. Surfactants are compounds with hydrophilic and hydrophobic properties that can help dissolve samples and seawater by lowering the surface tension and producing a more homogeneous solution without changing the chemical structure of the active compound (Olaru *et al*., 2025). The selection of 1% Tween 80 concentration in this study was based on the findings of Dos Santos *et al*. (2017), who reported that Tween 80 in the range of 0.5-1% was effective in increasing the solubility of ethanol extracts in the BSLT without having a significant toxic effect on Artemia salina larvae, thus not affecting the accuracy of the toxicity test results. In addition, a negative control containing seawater with Tween 80 was prepared without the addition of extracts. The addition of Tween 80 to the negative control ensured that the observed larval mortality was caused by the extract, not by the influence of the surfactant or the solvent medium itself (Olaru *et al*., 2025).

The microplate contained 10 shrimp larvae, and each experiment was replicated 3 times to minimize data errors. The larvae were observed 24 hours after the concentration treatment. Concentration. Larval mortality was calculated by observing the larvae for a few seconds. The standard criteria for assessing larval mortality *in A. salina* is when the larvae *do* not show movement for a few seconds of observation (Supriningrum, Sapri and Pranamala, 2016). Once the number of live larvae was known, the number of dead larvae was calculated. Calculate the percent of mortality at each concentration and control. Controls were used to correct for larval deaths that were not caused by the addition of extracts.

Table III. Toxicity Test Results of Ethanol Extract of Robusta Coffee Leaves

Concentration (ppm)	Concentration Log	Test Larvae Count	Number of Dead Larvae				% Deaths	Probit	LC50 (ppm)
			P1	P2	P3	Average			
1000	3,000	10	9	10	9	9,33	93	6,48	139,378
750	2,875	10	8	8	9	8,33	83	5,95	
500	2.699	10	7	7	6	6,67	67	5,44	
250	2.398	10	6	6	7	6,33	63	5,33	
100	2,000	10	5	4	5	4,67	47	4,92	
Control (-)	0	10	0	0	0	0	0	0	

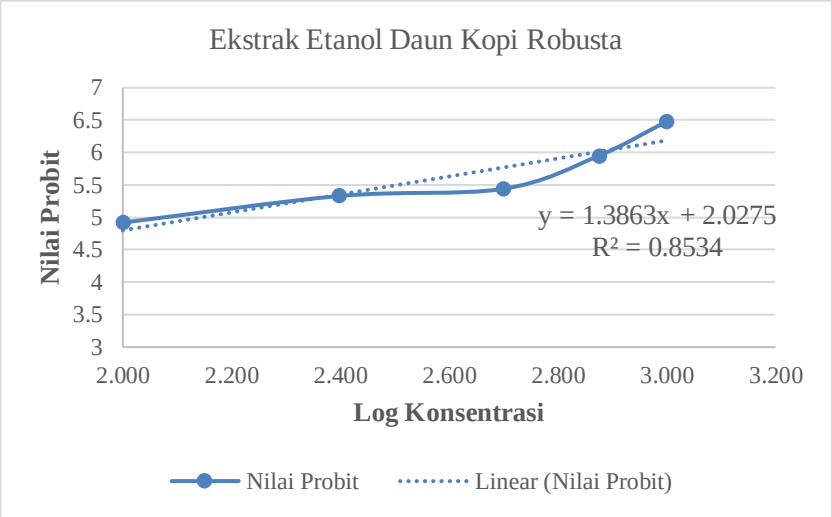
Information:
P = Repetition 1-3
LC50 0-100 ppm = high toxicity, LC_{50} 100-500 ppm = medium toxicity
LC50 500-1000 ppm = low toxicity, LC50 >1000 ppm = non-toxic

The results showed that larval mortality varied with different concentrations. The results are presented in **Table III**. Each treatment used 10 larvae with three replicates, resulting in a total of 30 larvae per concentration. Mortality rates ranged from 47% at the lowest concentration (100 ppm) to 93% at the highest concentration (1000 ppm), whereas the negative control showed no mortality. Variations in larval mortality across test bottles showed that different concentrations had different effects on *Artemia salina*. Higher concentrations of the extract led to greater larval mortality, confirming that the toxicity of the extract increased with increasing concentration.

After determining the percentage of larval mortality of A. *Salina*, the probit value was obtained from the probit table to calculate the Lethal Concentration 50 (LC50). LC50 shows the concentration needed to kill 50% of the test organisms. The LC50 value was calculated using a linear regression equation, in which the probit associated with 50% mortality was used as the y value to obtain x as the logarithm of the concentration. The antilog of x was then taken

as the value of LC50. This parameter serves as an indicator of the biological activity of a compound against the test organism, as it is determined by the number of larvae that die after exposure to a specific concentration of the compound (Saragih *et al.*, 2022).

The probit analysis on Robusta Coffee leaf extract obtained a straight line equation graph y=1.3863x+2.0275. After that, the y value is entered, which is the probit value of 50% of the test animal and the value of x = 2.14420 is obtained, then the LC50 antilog value is 139.378 ppm which is a moderate toxicity level. The range of medium toxic category is 100-500 ppm (Hamidi, Jovanova and Panovska, 2014). If the LC50 value of the tested extract or compound is <1000 ppm, it can be said that Robusta coffee leaf ethanol extract (*Coffea canephora*) in this experiment has a toxic potential for *Artemia salina* L. so that it has the potential for acute toxicity according to the BSLT method, namely in treatment with larval experimental animals *Artemia salina*, it also indicates the presence of biological activity so that this test can be used as an initial screening for bioactive compounds that are suspected to be efficacious as anticancers (Susanti *et al.*, 2023).



Picture 1. Graph of Regression Equations Between Concentration Logs and Prophytic Values

The mortality mechanism of Artemia salina larvae in the toxicity test is closely related to the function of secondary metabolites contained in robusta coffee leaves, including flavonoids, alkaloids, tannins, steroids, and phenolic compounds. These compounds act as antifedans by inhibiting the larvae's ability to eat. Flavonoids work by reducing the activity of digestive enzymes and the absorption of food, serving as stomach toxins that cause starvation and eventually dead larvae (Khasanah, Karyadi and Sundaryono, 2020). Alkaloids and tannins contribute to the death of larvae because they are active compounds that affect the nervous system, interfering with digestion by acting as oral toxins. This disorder makes the larvae unable to perceive taste stimuli so they cannot recognize their food, which results in starvation death (Source) *et al.*, 2014). Phenolic compounds at high concentrations act as plasma toxins by damaging cell walls and denatured cellular proteins, while at lower concentrations they inhibit the multiplication of enzymes in vitro, exerting toxic effects on other organisms by binding to proteins (Puspitasari *et al.*, 2018). In addition, steroids in certain amounts can also suppress appetite by affecting the nervous system involved in taste perception in the mouth area, reducing sensitivity and thus acting as an antifedan (Rahmawati *et al.*, 2024).

CONCLUSION

Ethanol extract of robusta coffee leaves (*Coffea canephora*) showed toxic activity to *Artemia salina* larvae in *the Brine Shrimp Lethality Test*, with an LC50 value of 139.378 ppm, indicating a moderate toxicity level (LC50 < 1000 ppm).

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