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Development of Microwave Maceration Method for the Extraction of Organic Constituents of Buton Bajakah (Kakatola) Root and Test of its Activity as an Antioxidant

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Abstract. The extraction of organic constituents, antioxidant activity test, and toxicity test of Bajakah Buton (Kakatola) root extract were conducted. Bajakah Buton roots were extracted using the microwave-assisted maceration method, followed by extraction using ethyl acetate solvent. The resulting yield reached 40,827% b/v. The analysis identified the presence of flavonoids, glycosides, phenols, terpenoids, and tannins. Antioxidant activity testing using the DPPH method showed IC_{50} values of ethyl acetate extract and vitamin C of 100.317 ppm and 13,797 ppm, respectively, indicating strong antioxidant properties. Toxicity tests using the BSLT method showed that the ethyl acetate extract of Bajakah Buton roots had a toxic activity with an LC_{50} value of 11,232 ppm. The results of this study will continue to be developed, so it is expected to be an important breakthrough in the field of cancer treatment.

Keywords: Antioxidants, Bajakah Buton, Maceration, Toxicity

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1. Introduction

Traditional medicine, which has been applied since ancient times, is a common practice in society. Hereditary experience recommends these methods for prevention, treatment and immune enhancement. Various degenerative diseases have emerged in the context of the development of science and technology. The focus on free radicals and antioxidants suggests that many degenerative diseases stem from the body's oxidation reactions. However, the body's antioxidant system plays a role in inhibiting free radical reactivity [1]. As a producer of medicinal plants, Indonesia has the Bajakah Buton (Kakatola) plant, which has potential as a medicine for degenerative diseases. Laboratory test results show that Bajakah has a high antioxidant content and 40 substances that have the potential to kill cancer cells, such as

saponins, phenolics, steroids, terpenoids, tannins, alkaloids, and terpenoids [2].

The maceration method is a technique for separating organic compounds in plants using organic solvents. In this study, water solvent was chosen as an alternative. This is based on the solubility of water by the extracted compounds, as well as consideration of the availability of water that is easily available [3]. The extraction method of Bajakah Buton (Kakatola) roots used is the microwave-assisted maceration method. Microwave-based extraction utilizes microwave heating in the extraction system, which has the advantage of obtaining extraction levels quickly. The maceration technique chosen involves soaking the powdered simplisia in an aqueous solution for several days at room temperature [4].

The 1-diphenyl-2-picrylhydrazyl (DPPH) assay method was used to assess the antioxidant activity of various samples, including compounds found in the Bajakah Buton (Kakatola) plant. This method provides information on the antioxidant capacity of the sample against DPPH, measured as the percentage of DPPH captured by the sample [5]. The higher the capture percentage, the stronger the antioxidant capacity of the sample. Compounds suspected of having anticancer activity are first tested on experimental animals using the Brine Shrimp Lethality Test (BSLT) method. BSLT testing is based on active compounds from plants that are toxic and able to kill Artemia salina shrimp larvae as test animals [6]. The results of toxicity tests with this method correlate with the cytotoxic power of anticancer compounds. Cytotoxic properties can be identified based on the number of larval deaths at a certain concentration [7].

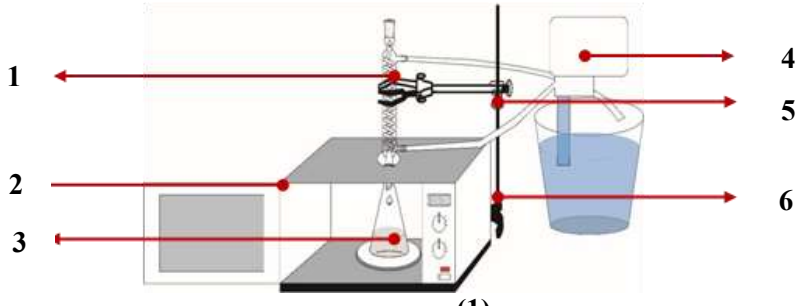
2. Methods

2.1. *Tools and Materials*

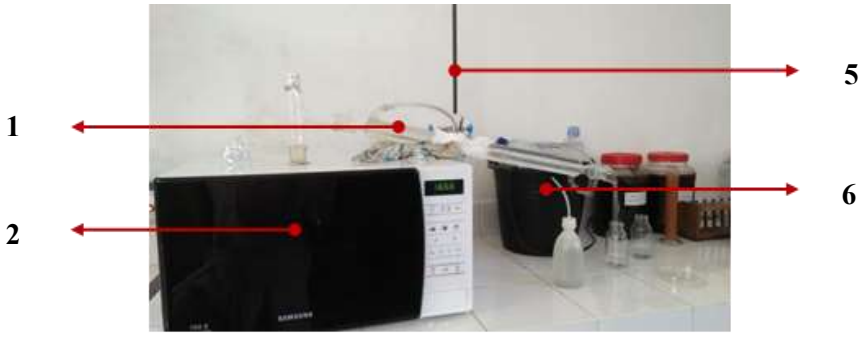
The tools used in this study are a microwave (Samsung), UV-Vis spectrophotometer (Jasco V-360) to measure the concentration of antioxidant compounds in a solution., blender (Panasonic), water pump, condenser, Erlenmeyer (pyrex), measuring cup (Pyrex), beaker, stirring rod, analytical balance (Explorer Ohaus), suction rubber (filler), volume pipette, capillary pipette, spray bottle, stopwatch, measuring pipette, volumetric flask (pyrex), drop pipette, funnel, stative, clamp, aluminium foil, scissors, knife, cloth, gloves, glass bottle, container bottle, vial bottle, ultraviolet lamp, bucket, sieve and petri dish. The materials used in this study are Bajakah Buton (Kakatola) root, Whatman filter paper, distilled water, technical n-hexane (C_6H_{14}), ethyl acetate ($CH_3CH_2OC(O)CH_3$), distilled water (H_2O), KLT plate, 5% hydrochloric acid (HCl), ascorbic acid ($C_6H_8O_6$), 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent 0,4%, Dragendroff reagent, Mayer reagent, acetone P, acetic acid P, sulfuric acid, sodium hydroxide, 10 mL ether P, 10% iron (III) chloride solution, chloroform and Artemia salina shrimp larvae.

2.2. Microwave Appliance Set

The microwave equipment consists of a microwave, sample container (Erlenmeyer), water pump, stative condenser, and clamps. The microwave is a Panasonic NN-ST342M type with a maximum power source of 800 W. The circuit of the microwave extraction apparatus is presented in Figure 1.



(1)



(2)

Descriptions:

1. Condenser
2. Microwave
3. Sample container (erlenmeyer)
4. Water Pump
5. Clamps
6. Stative

Figure 1. (1) Destruction Devices (2) Distillation Devices

2.3. *Maceration and Microwave Process*

Bajakah Buton (Kakatola) root powder (200 grams) was macerated with distilled water (3800 mL) for seven days. The extract was filtered to obtain a non-viscous maceration liquid. Next, Bajakah Buton (Kakatola) roots were re-extracted with 300 mL of water in an Erlenmeyer using a microwave (600 watts, 100 minutes) at 110 ^{0}C. The extracted macerate was stored and evaporated using microwave distillation to obtain a viscous macerate.

2.4. *Bajakah Buton (Kakatola) Root Water Macerate Extraction*

Bajakah Buton (Kakatola) root macerate was extracted using a separatory funnel, where each 50 mL of macerate was mixed with 50 mL of distilled solution with different polarities. After the separation of the two layers, the remaining maserat was mixed again with the distiller solution and repeated until clear. N-hexane and ethyl acetate distillation solutions were used to obtain extracts from the macerate. The extracts were evaporated with a rotary evaporator, and the yield was measured as the dry weight of Bajakah Buton (Kakatola) root extract.

2.5. *Qualitative analysis by KLT method*

A portion of the sample from each dilution was taken and blotted on a KLT plate with an n-hexane ethyl acetate mobile phase. After elution, it was dried, and the separation pattern was observed under UV light (254 nm - 366 nm). The Rf price was calculated and recorded at UV detection with the equation formula.

$$Rf = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ mobile\ phase}$$

2.6. Phytochemical screenings

Alkaloid Screening

The test solution was evaporated to form a residue. The residue was dissolved in 2 N HCl and divided into three tubes. Tube 1 was given dilute acid as a blank, tube 2 with Dragendorff reagent, and tube 3 with Mayer reagent. The formation of orange precipitate (tube 2) and yellow precipitate (tube 3) indicates the presence of alkaloids.

Flavonoid screening

The test solution was moistened with acetone, boric acid fine powder, and oxalic acid, which were added and heated in a water bath. The residue was mixed with ether and observed under UV light 366 nm. Yellow luminescence indicates the presence of flavonoids.

Saponin Screening

The test tube solution was shaken vertically for 10 seconds. The formation of 1-10 cm high foam that is stable for 10 minutes indicates the presence of saponins.

Tannin and polyphenol Screening

The test solution is divided into tube A (as blank), and tube B. Tube B reacts with 10% iron (III) chloride solution; dark blue or greenish black indicates tannins and polyphenols.

Glycoside Screening

The simplicia powder is dissolved in ethyl acetate and evaporated over a water bath, and the remainder is dissolved in anhydrous acetic acid with the addition of sulfuric acid. A blue or green colour indicates the presence of glycosides.

Steroid and triterpenoid Screening

The test solution was evaporated, and the residue was dissolved in chloroform. Anhydrous acetic acid and concentrated sulfuric acid were added to the solution. The formation of brownish or purple rings indicates the presence of triterpenoids, while blue-green rings indicate the presence of steroids.

2.7. Antioxidant Activity Test

The antioxidant test was conducted by incubating DPPH solution (blank), test solution + DPPH, and ascorbic acid + DPPH solution at 37°C for 30 minutes. The absorbance was measured at a wavelength of 517 nm with a UV-Vis spectrophotometer to obtain the inhibition value calculated with the following percentage equation.

$$\%\ resistance = \frac{Blank\ absorption - Sample\ absorption}{Blank\ absorption}\ x\ 100\%$$

2.8. Toxicity Test with the Brine Shrimp Lethality Test (BSLT) Method

Tests were conducted by injecting 100 µL of a solution containing 10-15 A. Salina Leach larvae into each well of the microplate, resulting in a variation of sample concentration. A no-sample control was also performed. Microplates were incubated for 24 hours at 22-29^{O}C. After that, the number of dead larvae was counted by looking at the wells using a flashlight, followed by the addition of 100 µL of methanol into the wells and left for 15 minutes. The LC_{50} value was obtained by calculating the percentage of death of test animals after 24 hours.

$$\%\ larvae = \frac{number\ of\ dead\ larvae}{number\ of\ test\ larvae}\ x\ 100\%$$

The next process involves finding the probit number through the probit table. A graph is created with the log of concentration on the x-axis and the percentage mortality in probit units on the y-axis. The LC50, which is the concentration that causes 50% mortality of the test animals, is calculated using the linear regression equation y = a + bx.

3. Results and Discussion

3.1. *Preparation and Extraction of Bajakah Buton (Kakatola) Root using microwave-assisted extraction (MAE)*

In this study, the roots of the Bajakah Buton (Kakatola) plant were washed and dried in the sun to reduce moisture content and prevent enzymatic reactions. The roots were pulverized into simplicia powder with a blender. The extraction process begins by wetting the powdered simplisia using an aqueous solvent to facilitate the penetration of the liquid into the pores of the simplisia. After maceration, the macerate is filtered to produce a liquid macerate, which is extracted using a microwave in a transparent glass reactor. Microwaves cause collision of water molecules, generate energy, and increase the temperature during irradiation [5]. After extraction, the simplicia are separated from the filtrate with filter paper. The blackish-brown filtrate contains water and bioactive compounds. A microwave was used to evaporate the solvent from the macerate, with the temperature maintained to keep the bioactive compounds functioning as antioxidants, resulting in a dark brown viscous macerate [8].

3.2. Liquid-Liquid Multistage Extraction

The yield of the macerate obtained was 6,725% (180 g thick extract from 200 g simplicia). Based on these results, the macerate was selected for liquid-liquid multistage extraction, resulting in two extract components. The extraction results show that the most Bajakah Buton (Kakatola) root extract is ethyl acetate extract (42.784% b/v), while the extract with n-hexane does not contain any compounds (0% b/v) (Table 1).

Table 1. Yield results of n-hexane and ethyl acetate extracts.

No.	Nama Ekstrak	% yield (% b/v)
1.	n-hexane	0
2.	Ethyl acetate	42,784

3.3. Qualitative analysis by KLT method

Thin Layer Chromatography (KLT) analysis on the extract was carried out using KLT plates eluted using a mixture of ethyl acetate and n-hexane in a ratio of 7:3. In the results of the analysis under UV light 254 nm, two stains were seen with Rf values of 0.1 and 0.46 respectively, as illustrated in Figure 2. The Rf value reflects the extent to which a compound moves on a KLT plate relative to its solvent. Compounds with smaller Rf values tend to be more retained by the solvent or migrate less over the KLT plate, while compounds with larger Rf values migrate more easily. Fluorescence under 254 nm UV light indicates there are at least two conjugated double bonds, while fluorescence under 365 nm UV light indicates the presence of longer conjugated double bonds called chromophores, with autochrome groups in the compound structure.

Figure 2. KLT profile of ethyl acetate extract of Bajakah Buton (Kakatola) root. A) under UV light 254 nm; B) under UV light 365 nm

3.4. Phytochemical Screening

Phytochemical screening was conducted on Bajakah Buton (Kakatola) root extract to identify secondary metabolites with potential antioxidant and anticancer activities as part of the toxicity test. The results of the phytochemical screening are documented in the table.

Table 2. Phytochemical screening results of ethyl acetate extract of Bajakah Buton (Kakatola) root

Phytochemical Test	**Positive Results According to Literature**	**Results**
	Formed orange precipitate (Dragendorff reagent)	
Alkaloid	Formed white precipitate (Mayer Reagent)	-
	Formed yellow precipitate (Wagner Reagent)	
Flavonoid	Discoloration of the control tube	++
Saponin	There is a foam that lasts ±10 minutes with a height of 10 cm.	-
Terpenoid	Brownish or violet rings	+

Tannin	A dark blue or greenish-black colour is formed.	++
Glycoside	The dark blue or green colour formed.	+
Polyphenol	A dark blue or greenish-black colour is formed.	++
Steroid	Formation of a blue-green ring	-

Description:

1. ++ sign: contained more compounds/concentrated colour
2. + sign: contained compounds/light colour
3. sign -: not contained compounds / no colour formed

Phytochemical screening of ethyl acetate extract of Bajakah Buton (Kakatola) roots identified secondary metabolite compounds such as flavonoids, glycosides, phenols, and tannins. In general, the secondary metabolites found were polar and soluble in the polar solvents used, namely water and ethyl acetate. Phytochemical screening of ethanol extract of Bajakah Tampala stem by maceration method showed the presence of flavonoids, saponins, terpenoids, tannins, phenols, and steroids without alkaloids [3]. In contrast, a study by Jabbar [8] stated that the ethanol extract of Bajakah Tampala from maceration contained alkaloids, flavonoids, and steroids. Susanto and Zayani's study [9] showed that Bajakah Tampala extract from maceration with methanol contained alkaloids, flavonoids, terpenoids, and phenolics. In conclusion, in general, Bajakah Buton (Kakatola) and Bajakah Tampala extracts have similar compounds.

3.5. Antioxidant Activity Test

Testing the activity of the Bajakah Buton (Kakatola) root extract was carried out by measuring the absorbance of each test solution using a UV-Vis Spectrophotometer at the maximum wavelength. Based on the research that has been done, the results of antioxidant activity testing of ascorbic acid standard solution are shown in Table 3.

Table 3. Antioxidant Activity Test Results of Ascorbic Acid

Concentration (ppm)	Average absorbance	Inhibition (%)	IC_{50} ppm
20	0,336	54,042	
40	0,288	60,657	
60	0,209	71,396	
			13,797
80	0,132	82,003	
100	0,071	90,283	
Blanko	0,731		

Based on the research that has been done, the results of antioxidant activity testing of Bajakah Buton (Kakatola) root extract are shown in Table 4.

Table 4. Antioxidant Activity Test Results of ethyl acetate root extract of Bajakah Buton (Kakatola)

Concentration (ppm)	Average absorbance	Inhibition (%)	IC_{50} ppm
20	0,604	17,381	
40	0,585	19,913	
60	0,499	31,775	100,317
80	0,436	40,397	
100	0,355	51,369	
Blanko	0,731		

Antioxidant activity testing on Bajakah Buton (Kakatola) root extract using ethyl acetate solvent and ascorbic acid standard solution was carried out with various concentrations whose absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. Based on the research that has been done, the relationship between variations in the concentration of standard solution and % inhibition can be seen in Figure 3.

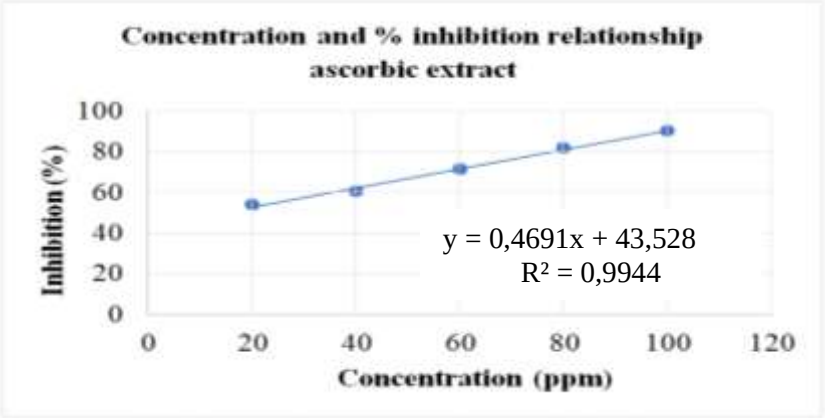


Figure 3: Graph of concentration relationship and % inhibition of ascorbic acid solution

Figure 3 shows the relationship curve between the concentration of ascorbic acid standard solution and antioxidant activity. The higher the concentration of ascorbic acid, the higher its silencing activity against free radicals. This is due to the increase in hydrogen atoms from the hydroxy group, which aids in the reduction of DPPH radicals to DPPH-H. Ascorbic acid, with two hydroxy groups, is more effective in donating hydrogen atoms. Based on the linear regression equation y = ax + b, the IC_{50} value for an ascorbic acid solution was 13,797 ppm. The relationship between concentration and % inhibition of Bajakah Buton (Kakatola) root extract can be seen in Figure 4.

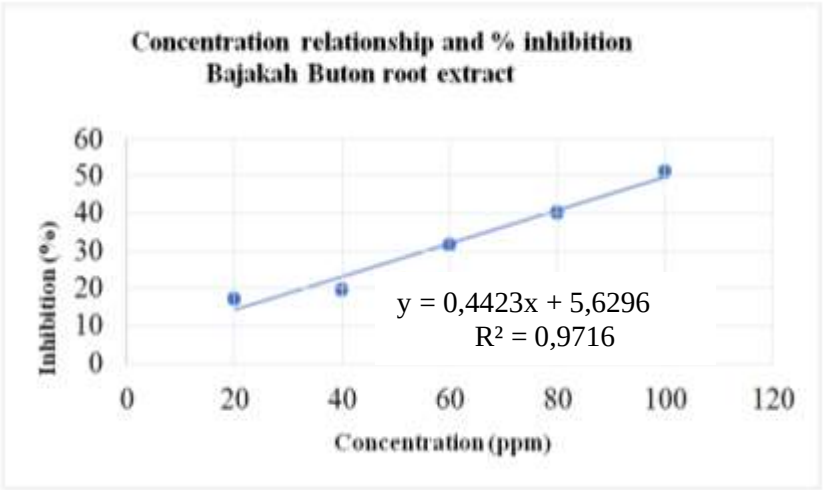


Figure 4. Concentration relationship curve and % inhibition of Bajakah Buton (Kakatola) root extract

Figure 4 displays the concentration curve of Bajakah Buton (Kakatola) root extract against antioxidant activity. The highest activity was at a concentration of 100 ppm, the lowest at 20 ppm. Increasing extract concentration increases secondary metabolite compounds, donating H atoms to DPPH radicals and forming stabilized DPPH-H. The more stabilized DPPH compounds, the colour intensity decreases the low absorbance value, increasing the percentage of antioxidant activity. The linear regression equation y = ax + b shows the IC_{50} of Bajakah Buton (Kakatola) root extract is 100,317 ppm, indicating high antioxidant power. This value indicates the concentration at which the antioxidant activity reaches 50%, and the result of the equation shows that the antioxidant power of the extract is rated as high. Therefore, Bajakah Buton (Kakatola) root extract has a fairly strong antioxidant power, which could have positive implications for the protection of cells from oxidative damage. According to Arysanti et al. [10], the IC_{50} value of a compound determines its antioxidant strength. $IC_{50} \leq 50$ ppm is considered very strong, 50-100 ppm as strong, 101-150 ppm as moderate, and > 151 ppm as weak. Based on the IC_{50} value, ascorbic acid is very strong because it is ≤ 50 ppm, purer and has two hydroxyl groups. Bajakah Buton (Kakatola) root extract is also strong with IC_{50} values between 0-100 ppm [11].

3.6. Acute Toxicity Test

The acute toxicity test in this study used the BSLT (Brine Shrimp Lethality Test) method with A. salina Leach shrimp larvae in a 96-well microplate. The number of samples used was minimal (0.6 mg) [12]. The A. salina larvae tested were 48 hours after hatching because, at the age of 24 hours, the mouth and digestive tract were not fully formed. Larvae aged 48 hours have a perfect mouth and digestive tract, as well as increased endurance [13]. Bajakah Buton root extract was prepared in seven concentration variations as the mother solution. A control without a sample was also prepared [14]. Larvae mortality data were processed using Excel analysis based on the Finney formula to determine the LC_{50} value with a confidence level of up to 95%. The results of the toxicity test of the Bajakah Buton (Kakatola) root extract can be found in Table 5.

Table 5. Results of BSLT Toxicity Test of Bajakah Buton (Kakatola) Root Extract

Concentration (ppm)	**Log C**	**Number of Live Larvae (Initial)**			**Total Dead Larvae**	**Larval Mortality (%)**	**LC_{50}**
X		1	2	3			
0 (Control)		10	10	10	0		
7,8125	0,89	10	10	10	5	16,7	
15,625	1,19	10	10	10	15	50	11,232 ppm (<1000 ppmor toxic)
31,25	1,49	10	10	10	30	100	
62,5	1,8	10	10	10	30	100	
125	2,1	10	10	10	30	100	
250	2,4	10	10	10	30	100	
500	2,7	10	10	10	30	100	

Figure 5 shows that the concentration of the extract affects the mortality rate of the test larvae, especially at a concentration of 500-31,25 µg/mL with the highest mortality rate. In the Brine Shrimp Lethality Test (BSLT), the level of toxicity is determined by the LC_{50} value, which is the concentration causing 50% mortality [15]. Compounds are considered active if they cause high mortality, with smaller LC_{50} values indicating greater mortality. The extract against A. salina Leach larvae is toxic if $LC_{50} \leq$ 1000 µg/mL, not toxic if $LC_{50} \geq 1000$ µg/mL. Toxicity is categorized as LC_{50} value ≥ 200 µg/mL and highly toxic if $LC_{50} \leq 30$ µg/mL [7].

The toxicity test of Bajakah Buton (Kakatola) root extract showed an LC_{50} of 11,232 ppm, indicating potential acute toxicity based on the BSLT method. This LC_{50} indicates that the concentration can cause 50% mortality of A. salina Leach larvae. In the context of potential use as an anticancer agent, a relatively high LC_{50} value indicates a low level of toxicity to the test organism. These findings support the potential of Bajakah Buton (Kakatola) root extract as an anticancer agent and motivate further research.

Tests were conducted on 48-hour-old A. salina Leach larvae when sensitivity to the test compounds reached maximum levels. Toxic compounds entering through oral and dermal routes cause changes in concentration gradients inside and outside the cell. Once absorbed into body tissues, the compounds invade cells, causing functional and metabolic damage to the larvae. The mechanism of larval mortality

is thought to involve these compounds, such as stomach toxins or gastric toxins, causing fatal damage to A. salina Leach cells.

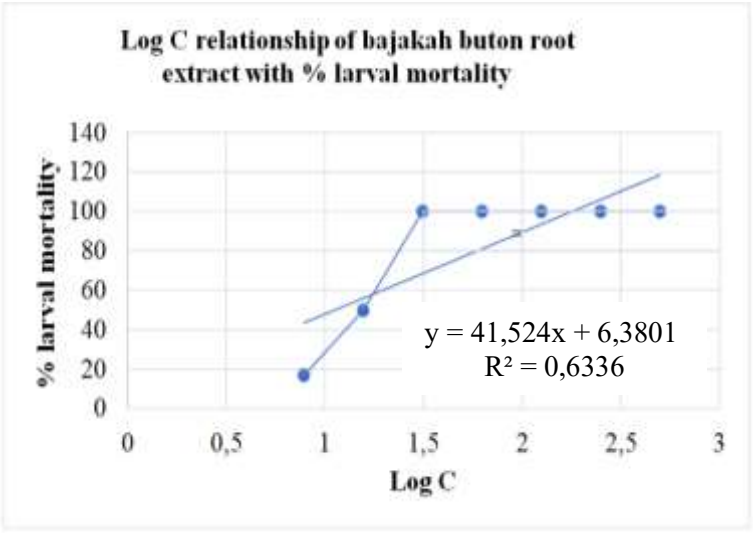


Figure 5: Log concentration curve of Bajakah Buton (Kakatola) root extract with % larval mortality

4. Conclusion

Phytochemical screening of ethyl acetate extract of Bajakah Buton (Kakatola) root identified flavonoids, saponins, glycosides, phenols, and tannins. DPPH antioxidant testing showed the IC_{50} values of the extract and vitamin C control were 100,317 ppm and 13,797 ppm, respectively, categorized as strong antioxidants (IC_{50} 0-100 ppm). An acute toxicity test using the BSLT method showed toxic activity (LC < 1000 ppm) with LC_{50} 11,232 ppm. This research will continue to be developed, so it is expected to be an important breakthrough in the field of cancer treatment [16].

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