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Extraction of The Chemical Components of Dengen Leaves (*Dillenia serrata* Thunb) by MAE Method and Activity Test as Antioxidant and Toxicity

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Abstract

Research on the extraction of chemical components of Dengen (Dillenia serrata Thumb) leaves using the MAE (microwave-assisted extraction) method and activity as an antioxidant and toxicity test has been carried out. This study aimed to extract the chemical components of Dengen leaves using the MAE method and to test the antioxidant activity and toxicity of the ethanol extract of Dengen leaves. The chemical components of Dengen leaves were extracted by the MAE method and obtained ethanol extract with a yield of 47%. Dengen leaves ethanol extract was partitioned with n-hexane and ethanol as solvents and obtained yields of 5% (n-hexane) and 65% (ethanol). The chemical components of Dengen leave ethanol extract were identified by phytochemical screening. The results of phytochemical screening showed the presence of secondary metabolites of alkaloids, flavonoids, saponins, polyphenols, terpenoids, and steroids. The antioxidant activity test of the ethanol extract of Dengen leaves was carried out using the DPPH (2,2-diphenyl-1-picrihydrazil) method and obtained the value of IC50 = 100,363 ppm (strong antioxidant). A toxicity test of the ethanol extract of Dengen leaves was carried out using the BSLT (Brine Shrimp Lethality Test) method and obtained the value of LC50 = 18.3443 ppm (very toxic).

Keywords: Antioxidant, Dillenia serrata, Microwave, Extraction, Toxicity.

INTRODUCTION

Dengen plant (Dillenia serrata Thumb) is one of the endemic plants that grow wild in Sulawesi. Dengen plant is used in traditional medicine. Dengen bark decoction is used as a medicine for vomiting blood Windardi et al., 2006; Sinala et al., 2021. Dengen leaf decoction is used as a remedy for indigestion. Dengen fruit juice is used as a thrush medicine (Purnawati et al., 2020). For these traditional medicinal raw materials to be medically accountable, it is necessary to conduct scientific testing on chemical components, efficacy, safety, and quality standards.

Initial information about the chemical components of a plant can be obtained through extraction and phytochemical screening. The method commonly used to extract the chemical components of a plant is maceration. The advantages of the maceration method are that it is easier, simpler, and cheaper to process. Another advantage of maceration is that this method can be used to extract medicinal substances from a heat-resistant and non-heat-resistant simplicia because this method is carried out at room temperature. The disadvantages of the maceration method are that the time required to extract the material is quite long, the extraction is not perfect or not optimal, and the volume of solvent used is quite a lot if we have to do maceration.

The disadvantages of the maceration method can be overcome by using the MAE (microwave-assisted extraction) method. The MAE method is a technique for extracting dissolved materials in a simplicia with the help of microwave energy. This technology is suitable for the extraction of thermolabile compounds

because this method has better temperature control than conventional heating processes. Other advantages of the MAE method are shorter extraction times, less energy and solvent usage, higher yields, higher accuracy and precision, improved mass transfer, and equipment settings that incorporate both soxhlet and microwave features (Fadiyah et al., 2020).

Phytochemical screening is a preliminary test carried out to determine secondary metabolites contained in a plant. The content of secondary metabolites of the Dengen plant that have been reported by researchers are alkaloids, flavonoids, saponins, polyphenols, tannins, terpenoids, and steroids. These secondary metabolites have been reported to be contained in the methanol extract of Dengen bark (Jalil et al., 2015), ethanol extract of Dengen fruit (Illing et al., 2017), and ethanol extract of Dengen leaves (Purnawati et al., 2020).

The efficacy of extracts or secondary metabolites of the Dengen plant can be seen from the results of the bioassay test. One of the commonly used bioassay tests is the antioxidant activity test using the DPPH (1,1-diphenyl-2-picrylhydrazil) method. The parameter used to determine the antioxidant activity of a compound or extract is IC50 (Inhibition Concentration 50%). The IC50 value indicates the concentration of the compound or extract that can reduce the absorption intensity or counteract DPPH free radicals by 50%. The part of the Dengen plant that has been reported to have antioxidant activity is fruit juice with an IC50 value of 161.63 mg/mL (Purba & Mujadilah, 2017).

The way to find out if a plant contains bioactive compounds is to do a toxicity test. This test is a preliminary test to determine the pharmacological activity of a compound or extract. One of the methods that can be used for toxicity test is BSLT (Brine Shrimp Lethality Test). The parameter used to determine the level of toxicity of a compound or extract is LC_{50} (Lethal Concentration 50%). The LC_{50} value indicates the concentration of toxic substances that can cause the death of test animals up to 50%. So far, no part of the Dengen plant has been reported to be toxic. Therefore, it is important to conduct this research to determine the chemical components of the ethanol extract of Dengen leaves which have the potential as raw materials for anticancer drugs.

METHODOLOGY

Materials and Instrumentals

The materials used are Dengen leaves (*Dillenia serrata* Thunb), ethanol (Technical), n-hexane (Technical), ethyl acetate (Technical), aquabidest (Onelab), aluminum foil (Diamond), tissue (Nice), filter paper (whatman), $HgCl_2$ p.a. (E. Merck), KI p.a. (E. Merck), H_2SO_4 p.a. (E. Merck), I_2 p.a. (E. Merck), $FeCl_3$ p.a. (E. Merck), $Bi(NO_3)_3$ p.a. (E. Merck), HNO_3 p.a. (E. Merck), CH_3CO_2H p.a. (E. Merck), HCl p.a. (E. Merck), Mg p.a. (E. Merck), $CHCl_3$ p.a. (E. Merck), CH_3OH p.a. (E. Merck), acetic anhydride p.a. (E. Merck), vitamin C or ascorbic acid ($C_6H_8O_6$) p.a. (E. Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, Meyer reagent, Wagner reagent, Wilstater reagent, Liebermann-Burchad reagent, and shrimp larvae *Artemia salina* Leach.

The instrumentals used are analytical balance (Scientech), blender (sharp), chamber (Pyrex), chemical beaker (Pyrex), cutter (Bazic), dropper pipette (Pyrex), Electrothermal 9100, Erlenmeyer (Pyrex), funnels (Pyrex), heating mantles, jar, magnetic stirrers (1 cm), measuring cup (Pyrex), multichannel micropipette (30-300, 20-200, 10-100 μL), 96-well microplate, microwave (Panasonic NN-ST342M), pump air (Shimizu), porcelain cup, refrigerator (Sharp), rotary vacuum evaporator (Buchi Germany), ruler (Butterfly), scissor (Joyko), UV-Vis spectrophotometer (Jasco V-360), stirring rod, thermometers (100^{o}C), and vacuum oven (Cosmos).

Methods

Microwave Assisted Extraction (MAE)

Dengen leaves powder (500 g) was macerated with ethanol (1500 mL) for 3 x 24 hours. Every 24 hours, the macerate was filtered to separate the filtrate and residue. The filtrate was stored in a jar and the residue was macerated again with ethanol (1500 mL). The filtrate obtained from each filtration is combined into one. The filtrate (300 mL) was put into an Erlenmeyer flask in the microwave. The filtrate was destroyed by microwave at 600 watts for 100 minutes. The digested filtrate is stored in a jar. This procedure was repeated until all the filtrate was destroyed in the microwave. The ethanol liquid macerate from the destruction was combined and concentrated with a rotary vacuum evaporator at 78^{o}C. Dengen leaves concentrated ethanol extract was weighed and the yield was calculated (Fadiyah et al., 2020).

Partition

Dengen leaves concentrated ethanol extract (100 g) was dissolved in ethanol (200 mL). Dengen leaves ethanol extract solution (50 mL) was put into a separating funnel and partitioned in stages with n-hexane and ethanol as solvents. This procedure was

repeated until all the ethanol extract solution of Dengen leaves was partitioned in stages.

Phytochemical Screening

Alkaloid Test

Dengen leaves concentrated ethanol extract (1 mg) was dissolved in ethanol (5 mL). Dengen leaves ethanol extract solution (2 mL) was added with 2 N HCl (1 mL) and aquabidest (3 mL). The solution was heated while stirring with a magnetic stirrer for 3 minutes. The sample solution (1 mL) was put into 2 different test tubes. Meyer's reagent (3 drops) was added to test tube 1. The alkaloid test was declared positive if a yellowish white or cream precipitate was formed. Wagner reagent (3 drops) was added to test tube 2. The alkaloid test was declared positive if a reddish brown or orange precipitate was formed (Jaafar et al., 2007; (Fadeyi et al., 2022).

Flavonoid Test

Dengen leaves concentrated ethanol extract (1 mg) was dissolved in ethanol (5 mL). Dengen leaves ethanol extract solution (2 mL) was added with Mg powder (1 mg) and concentrated HCl (1 mL). The mixture was heated and stirred with a magnetic stirrer until the Mg powder dissolved. The flavonoid test was declared positive if a yellowish red or orange color was formed (Jaafar et al., 2007; Souhoka et al., 2019; Fadeyi et al., 2022).

Saponin Test

Dengen leaves concentrated ethanol extract (1 mg) was dissolved in ethanol (5 mL). Dengen leaves ethanol extract solution (2 mL) was added with hot aquabidest (1 mL) and concentrated HCl (3 drops) and then shaken for 30 seconds. The saponin test was declared positive if a stable foam was formed for 1 minute (Jaafar et al., 2007; Yanti et al., 2021; Fadeyi et al., 2022).

Polyphenol Test

Dengen leaves concentrated ethanol extract (1 mg) was dissolved in ethanol (5 mL). Dengen leaves ethanol extract solution (2 mL) was added with 10% $FeCl_3$ (3 drops). The polyphenol test was declared positive if a blue-black color was formed (Jaafar et al., 2007; Fadeyi et al., 2022).

Terpenoid and Steroid Test

Dengen leaves concentrated ethanol extract (1 mg) was dissolved in ethanol (5 mL). Dengen leaves ethanol extract solution (2 mL) and $CHCl_3$ (1 mL) were put into 2 different test tubes. Liebermann-Burchad reagent (1 mL) was added slowly through the wall of the test tube. The terpenoid test was declared positive if a brown or violet ring is formed at the solution boundary. The steroid test was declared positive if a greenish-blue ring was formed at the solution boundary (Jaafar et al., 2007; Fadeyi et al., 2022).

Antioxidant Activity Test with DPPH Method

DPPH solution (blank) (1 mL), test solution (20, 40, 60, 80, 100 μg/mL) (4 mL) + DPPH 0.3 mM (1 mL), and ascorbic acid solution (20, 40 , 60, 80, 100 μg/mL) (4 mL) + DPPH 0.3 mM (1 mL) were incubated at 37^{o}C for 30 minutes. The absorbance of each solution was measured by UV-Vis spectrophotometer at a wavelength of 510-520 nm (Marliani et al., 2015; Dali et al., 2013; Dali et al, 2017; Souhoka et al., 2019; Yanti et al., 2021). The percentage of inhibition was calculated using Equation (1).

$$\% \text{ Inhibition} = \frac{(A_{blank}) - (A_{sample})}{(A_{blank})} \text{ x } 100\% \quad (1)$$

Inhibitory Concentration 50% (IC_{50}) of Dengen leaves ethanol extract and ascorbic acid (vitamin C) was determined from the graph of concentration (ppm) as the x-axis against the percentage of inhibition (%) as the y-axis. The IC_{50} value is obtained from the value of x (concentration) after replacing the value of y = 50 (inhibition 50%) in the linear regression equation, y = a + bx.

Antioxidant Activity Test Criteria

A substance is said to be very strong antioxidant if the value ($IC_{50} \leq 50$ ppm), strong (IC_{50} between 51-100 ppm), moderate (IC_{50} between 101-150 ppm), weak (IC_{50} between 151-200 ppm), and very weak ($IC_{50} \geq 201$ ppm). (Kedare & Singh, 2011)

Toxicity Test with BSLT Method

A toxicity test was carried out using the Brine Shrimp Lethality Test (BSLT) method. The test was carried out by entering the test solution (100 μL) (7.8125, 15.625, 31.25, 62.5, 125, 250, 500 ppm) containing *Artemia salina* Leach shrimp larvae (10-15 tails) into each well on a microplate. The microplate was incubated for 2 x 24 hours at a temperature of 22-29^{o}C. The number of live and dead larvae was counted every 24 hours. This procedure is repeated up to three times. While the control or without the addition of the test solution was only carried out once (Meyer et al., 1982; Solis et al., 1992; (Carballo et al., 2002); McLaughlin et al., 1998; Solanki et al., 2013; Mentors et al., 2014).

Percentage of mortality and corrected mortality of *Artemia salina* Leach shrimp larvae were calculated using Abbott's Equations (2) and (3) (Finney, 1952; Finney 1971).

$$\% \text{ Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of live larvae}} \text{x} 100\% \quad (2)$$

$$\text{Corrected mortality } (\%) = \frac{M_{obs} - M_{control}}{100 - M_{control}} \text{ x } 100 \quad (3)$$

The Lethal Concentration 50% (LC_{50}) value was calculated using a graph or regression method according to the standard probit analysis procedure. How to calculate LC_{50} using the graphical method, namely from the graph of the probit value (y-axis) to the concentration of $\log_{10}$ (x-axis) a straight line (linear) is drawn through the plotted points. This straight line is used to estimate the concentration of $\log_{10}$ (x) associated with the value of probit (y) = 5 (probit 50%). The LC_{50} value was obtained by changing the concentration value of $\log_{10}$ (x) to antilog. Meanwhile, how to calculate LC_{50} using the regression method, namely the concentration value of $\log_{10}$ (x) is calculated from the linear regression formula, y = a + bx, which is related to the value of probit (y) = 5 (probit 50%). The LC_{50} value is obtained by changing the concentration value of $\log_{10}$ (x) to antilog (Finney, 1952; Finney 1971).

Toxicity Testing Criteria

The toxicity of herbal extracts expressed as LC_{50} values is commonly valorized either by comparison to Meyer's or to Clarkson's toxicity index. According to Meyer's toxicity index, extracts with LC_{50} < 1000 µg/mL are considered as toxic, while extracts with LC_{50} > 1000 µg/mL are considered as non-toxic (Meyer et al., 1982). Clarkson's toxicity criterion for the toxicity assessment of plant extracts classifies extracts in the following order: extracts with LC_{50} above 1000 µg/mL are non-toxic, LC_{50} of 500 - 1000 µg/mL are low toxic, extracts with LC_{50} of 100 - 500 µg/mL are medium toxic, while extracts with LC_{50} of 0 - 100 µg/mL are highly toxic (Clarkson et al., 2004).

RESULTS AND DISCUSSION

Extraction and Partition

The results of microwave-assisted extraction of Dengen leaves powder in ethanol solvent and the results of liquid-liquid partitioning of Dengen leaves ethanol extract with n-hexane and ethanol as solvents are shown in Table 1. Extraction is the process of withdrawing the components of bioactive compounds from a mixture of solids or liquids using certain solvents. The components of the bioactive compounds in the mixture will move into the solvent during the extraction process. The extraction method used in this research is MAE. MAE method is an extraction process that utilizes the energy generated by microwaves in the form of electromagnetic radiation. The extraction process takes place in a glass reactor which is irradiated with microwaves at a voltage of 600 watts for 100 minutes. The glass reactor used is clear. This is so that the microwaves can penetrate the walls of the glass reactor so that they can interact with solvent molecules. The solvent molecules will move randomly during the microwave irradiation process, causing collisions between solvent molecules. This successive molecular collision will generate energy, so that the temperature in the glass reactor increases. The heat generated by the collision of these molecules will destroy the cell walls of the simplicia, so this process will help the mass transfer of the simplicia bioactive compounds into the solvent. This process causes the weight of crude (235 g) (Table 1) obtained from the MAE method of Dengen leaves in ethanol solvent to be heavier than the extraction results obtained from the conventional maceration method (145 g) (Purnawati et al., 2020).

Fractionation is the process of separating mixed components of an extract based on differences in polarity properties. The fractionation method used in this research is liquid-liquid partition. Liquid-liquid partitioning is done by adding n-hexane as a solvent in the extract which has been dissolved in ethanol so that two phases are formed. The components of the bioactive compounds in the extract will dissolve between the two phases according to their polarity. The process of dissolving substances in this solvent is by the principle of like dissolves, i.e. the solubility of a compound between two phases will depend on the similarity of the polarity of the compound with the liquid solvent. This process causes the weight of crude obtained from the liquid-liquid partition extraction of Dengen leaves ethanol extract in ethanol solvent (65 g) to be heavier than in n-hexane (5 g) solvent (Table 1).

Phytochemical Screening

The results of phytochemical screening of the ethanol extract of Dengen leaves are shown in Table 2. Table 2 shows that the ethanol extract of Dengen leaves contains secondary metabolites of alkaloids, flavonoids, saponins, polyphenols, terpenoids, and steroids. Secondary metabolites of this group have also been found in Dengen leaves (Purnawati et al., 2020), Dengen fruit (Bandara et al., 2015); (Gandhi &

Mehta, 2013); Suaib, 2021; Illing et al., 2017; Illing et al., 2018; Illing et al., 2019;), and Dengen bark (Sabandar et al., 2020).

the more antioxidant compounds react with DPPH free radicals.

On the other hand, the higher the concentration

Table 1. The results of extraction and partition of ethanol extract of Dengen leaves

Extraction Method	Sample Weight (g)	Solvent	Solvent Volume (mL)	Crude Weight (g)	Results (%)
Microwave assisted extraction (MAE)	500	Ethanol	4500	235	47
Liquid-liquid partition	100	n-Hexane	200	5	5
		Ethanol	200	65	65

Table 2. The results of phytochemical screening of ethanol extract of Dengen leaves

Phytochemical Test	Reagent	Discoloration		Observation result	Test results
		Before	After		
Alkaloids	Meyer			A yellowish white or cream precipitate is formed	+
	Wagner			A reddish brown or orange precipitate is formed	+
Flavonoids	Wilstater			Formation of yellowish red or orange color	++
Saponins	Hot Aquabidest + concentrated HCl			Stable foam is formed for 1 minute	+
Polyphenol	$FeCl_3$			Formation of dark blue color	++
Terpenoids	Liebermann-Burchad (LB)			A reddish brown ring is formed at the solution boundary	++
Steroids	Liebermann-Burchad (LB)			A blue-green ring is formed at the solution boundary	++

Description: (-) = negative; (+) = weak positive; (++) = strong positive

Antioxidant Activity Test with DPPH Method

The results of the average absorbance measurement at λ_{max} (513 nm), calculation of % inhibition, and IC_{50} of ethanol extract of Dengen leaves and ascorbic acid (vitamin C) are shown in Table 3. The percentage of inhibition is a parameter used to indicate the concentration of an antioxidant compound in inhibiting DPPH free radicals. Table 3 shows that the higher the concentration of ethanol extract of Dengen leaves, the higher the percentage of free radical inhibition of DPPH. These results indicate that the higher the concentration of the test sample, of Dengen leaves ethanol extract, the lower the absorbance value. This is indicated by a change in the intensity of the color of the solution, i.e. dark purple from DPPH free radicals changes to yellow from neutral DPPH-H at λ_{max} (513 nm). This phenomenon is also seen in the absorbance value and the percentage of DPPH free radical inhibition of vitamin C as a comparison. However, the absorbance value of the ethanol extract of Dengen leaves > vitamin C and the percentage of free radical inhibition of DPPH from the ethanol extract of Dengen leaves < vitamin

C. This is because vitamin C is a relatively purer compound than the ethanol extract of Dengen leaves.

Table 3. The results of the average absorbance measurement at λ_{max} (513 nm), calculation of % inhibition, and IC_{50} of ethanol extract of Dengen leaves and ascorbic acid (vitamin C)

Concentration (ppm)	Dengen leaves ethanol extract			Ascorbic acid (vitamin C)		
	Average Absorbance	Inhibition (%)	IC_{50} (ppm)	Average Absorbance	Inhibition (%)	IC_{50} (ppm)
20	0.719	4.52		0.659	12.48	
40	0.635	15.67		0.537	28.69	
60	0.573	23.90	77.3269	0.415	44.89	62.6396
80	0.391	48.07		0.273	63.75	
100	0.157	79.15		0.091	87.92	
Blank	0.753	-	-	0.753	-	-

The graph of the relationship between the concentration of Dengen leaves ethanol extract and vitamin C added to DPPH with the percentage of free radical inhibition of DPPH is shown in Figures 1 and 2. The value of x (IC_{50}) for the ethanol extract of Dengen leaves can be obtained from the linear regression line equation y = 0.9083x - 20.236 (Figure 1). If the value of y = 50 (inhibition 50%), the value of x (IC_{50}) = 77.3269 ppm (Table 3). The value of x (IC_{50}) for vitamin C can be obtained from the equation of the linear regression line y = 0.9297x - 8.236 (Figure 2). If the value of y = 50 (inhibition 50%), the value of x (IC_{50}) = 62.6396 ppm (Table 3). Thus, the IC_{50} value of the ethanol extract of Dengen leaves (77.3269 ppm) was greater than the IC_{50} of vitamin C (62.6396 ppm).

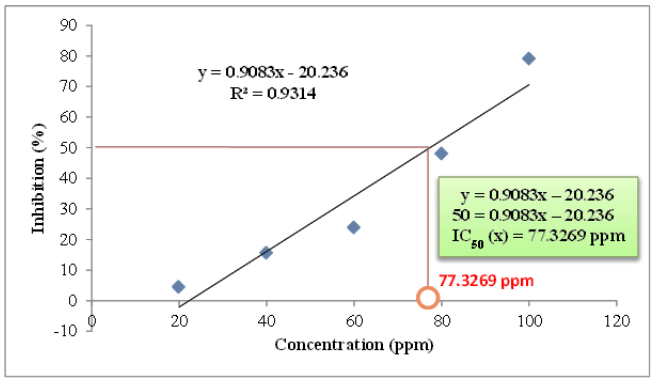

Figure 1. Graph of the relationship between the concentration of ethanol extract of Dengen leaves + DPPH with the percentage of free radical inhibition of DPPH.

IC_{50} value is a parameter used to indicate the concentration of an antioxidant compound that can inhibit 50% of DPPH free radicals. The smaller the IC_{50} value of a compound, the higher its antioxidant activity. A compound is said to have very strong antioxidant activity if the value is ($IC_{50} \leq 50$ ppm), strong ($51 \leq IC_{50} \leq 100$ ppm), moderate ($101 \leq IC_{50} \leq 150$ ppm), weak ($151 \leq IC_{50} \leq 200$ ppm), and very weak. ($IC_{50} \geq 201$ ppm) (Molyneux, 2004); (Dali et al., 2017). Therefore, the antioxidant activity of the ethanol extract of Dengen leaves was strong (IC_{50} = 77.3269 ppm) (Table 3) (Figure 1). Similarly, the antioxidant activity of vitamin C was included as strong (IC_{50} = 62.6396 ppm) (Table 3) (Figure 2). The results of the research by Irnawati et al., (2017) also showed that the antioxidant activity of vitamin C was very strong (IC_{50} = 24.63 mg/L) and Dengen fruit juice was weak (IC_{50} = 161.63 mg/L). Meanwhile, the results of the research by Sabandar et al., (2020) showed that the methanol extract of Dengen stem bark could capture DPPH free radicals with an inhibition percentage of 48.2 - 59.7% compared to vitamin C, trolox, and gallic acid of 90.3 - 93.8% at a concentration of 100 µg/mL.

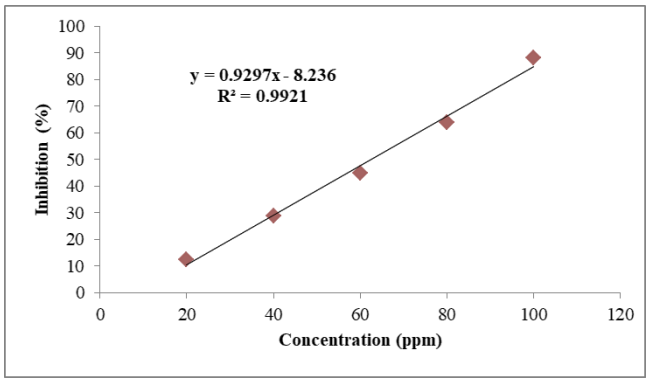

Figure 2. Graph of the relationship between the concentration of vitamin C + DPPH with the percentage of free radical inhibition of DPPH.

The antioxidant activity of a compound is measured by its ability to scavenge free radicals. The results showed that the antioxidant activity of the ethanol extract of Dengen leaves was strong (IC_{50} = 77.3269 ppm) (Table 3) and (Figure 1). This means that the ethanol extract of Dengen leaves is quite strong in inhibiting the free radical reaction of DPPH. The mechanism of inhibition of this reaction is that DPPH free radicals capture H atoms from the

bioactive components of the ethanol extract of Dengen leaves to form DPPH-H. This reaction causes a change in the color of the solution from dark purple to yellow at λ_{max} (513 nm) (Figure 3) (Nurhasnawati et al., 2017); Dali et al., 2017).

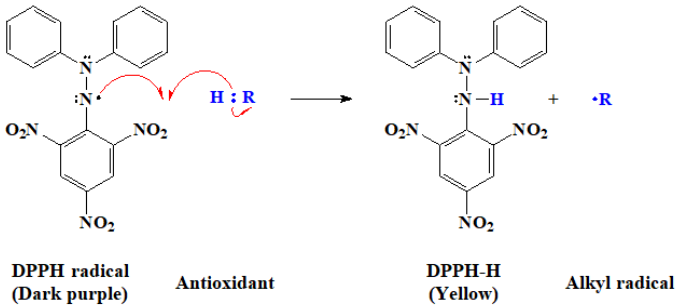

Figure 3. DPPH free radical scavenging reactions by antioxidants

Toxicity Test with BSLT Method

Data on the results of the toxicity test of the ethanol extract of Dengen leaves on *Artemia salina* Leach shrimp larvae are shown in Table 4. Table 4 shows that the higher the concentration of ethanol extract of Dengen leaves, the higher the mortality rate of *Artemia salina* Leach shrimp larvae. These results indicate that there is a positive correlation between the concentration level of Dengen leaves ethanol extract and the mortality percentage of *Artemia salina* Leach shrimp larvae. This is because the higher the concentration of the ethanol extract of Dengen leaves, the more the amount of bioactive compounds contained in it, so the ability of the bioactive compounds to kill *Artemia salina* Leach shrimp larvae is also higher. Thus, the higher the concentration of ethanol extract of Dengen leaves, the higher its ability to kill *Artemia salina* Leach shrimp larvae.

The level of toxicity of the ethanol extract of Dengen leaves on *Artemia salina* Leach shrimp larvae can be determined from the Lethal Concentration 50% (LC_{50}) value. LC_{50} is a value that indicates the concentration of toxic substances that can cause the death of test larvae up to 50%. The LC_{50} value of the ethanol extract of Dengen leaves can be calculated from the data on $\log_{10}$ concentration and corrected mortality of *Artemia salina* Leach shrimp larvae in Table 4 using probit analysis at a 95% confidence level (Finney, 1952; Finney, 1971).

The graph of the relationship between the $\log_{10}$ concentration of Dengen leaves ethanol extract and the percentage of mortality corrected by *Artemia salina* Leach shrimp larvae in probit units is shown in Figure 4. The x value ($\log_{10}$ concentration) for Dengen leaves ethanol extract can be obtained from the linear regression line equation y = 2.9934x + 1.2179 (Figure 4).

Table 4. The results of the toxicity test of the ethanol extract of Dengen leaves on *Artemia salina* Leach shrimp larvae

Concentration (C) (ppm)	$\log_{10}$ C	Number of Live Larva			Number of Dead Larva	Mortality (%) (48 hours)	Corrected Mortality (%)	Probit	LC_{50} (ppm)
		1	2	3					
Control (0.0)	-	10	10	10	1	3.33	-	-	
7.8125	0.893	10	10	10	5	16.67	14	3.92	
15.625	1.194	10	10	10	11	36.67	35	4.62	
31.25	1.495	10	10	10	25	83.33	83	5.95	18,3443
62.5	1.796	10	10	10	28	93.33	93	6.48	
125	2.097	10	10	10	30	100	100	-	
250	2.398	10	10	10	30	100	100	-	
500	2.699	10	10	10	30	100	100	-	

If the value of probit (y) = 5 (the probit of 50%), the value of $\log_{10}$ concentration (x) = 1.2635 is obtained. So, LC_{50} = antilog (1.2635) = 18.3443 ppm (Table 4) and (Figure 4). The LC_{50} value is a parameter used to indicate the concentration of an extract or compound that can cause a test larvae mortality rate of 50%. A compound or extract is said to be toxic if it produces a high percentage of mortality. The smaller the LC_{50} value of a compound or extract, the greater the percentage of mortality (Mentor et al., 2014). A compound or extract is said

to be toxic if $LC_{50} \leq 1000$ ppm and non-toxic if $LC_{50} \geq 1000$ ppm (Meyer et al., 1982). An extract or compound is said to be non-toxic if $LC_{50} \geq 1000$ ppm, low toxic if LC_{50} of 500 - 1000 ppm, moderately toxic if LC_{50} of 100 - 500 ppm, and very toxic if LC_{50} of 0 - 100 ppm (Clarkson et al., 2004).

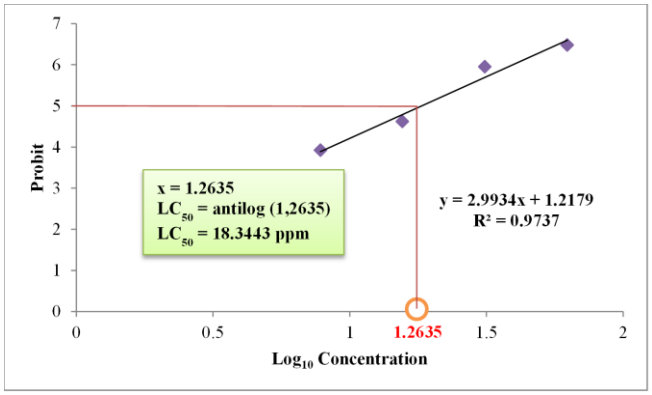

Figure 4. Graph of the relationship between log_{10} concentration of Dengen leaves ethanol extract and percent mortality corrected by *Artemia salina* Leach shrimp larvae in probit units

The results of the toxicity test on the ethanol extract of Dengen leaves showed the value of LC_{50} = 18.3443 ppm (Table 4) and (Figure 4). These results indicate that the ethanol extract of Dengen leaves is highly toxic (value of LC_{50} = 18.3443 ppm 100 ppm) (Clarkson et al., 2004). Thus, the ethanol extract of Dengen leaves has the potential for acute toxicity according to the BSLT method, so it can be developed as an anticancer agent. The potential for acute toxicity of the ethanol extract of Dengen leaves is influenced by the content of its secondary metabolites.

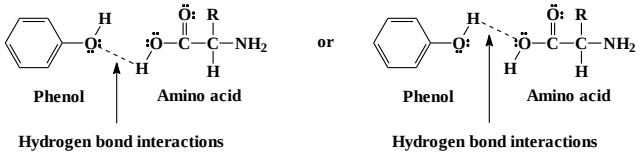

Figure 5. OH group of phenol interacting by hydrogen bonding with OH group of the side chain of carboxylic acid from amino acid residues of protein (Matta et al., 1996)

One of the secondary metabolites contained in the ethanol extract of Dengen leaves is polyphenol (Table 2). Polyphenols are polymers of high molecular weight phenols. Phenol is a compound that has a hydroxyl group attached directly to the benzene ring. The phenol OH group from the ethanol extract of Dengen leaves can interact through hydrogen bonding with the OH group of the carboxylic acid side chain of the amino acid residue of protein (Figure 5).

The formation of this hydrogen bond (Figure 5) causes one amino acid to be unable to react with another amino acid to form a protein, so synthesis is inhibited (Figure 6). In addition, the formation of hydrogen bonds (Figure 5) also causes the active transport of Na^+ and K^+ ions into the cell membrane to stop (Matta et al., 1996). As a result, the entry of Na^+ and K^+ ions into the cell membrane is not controlled, so cell membrane breaks or is denatured. This process caused the death of *Artemia salina* Leach shrimp larvae during the incubation period because the nutrient transport route was interrupted.

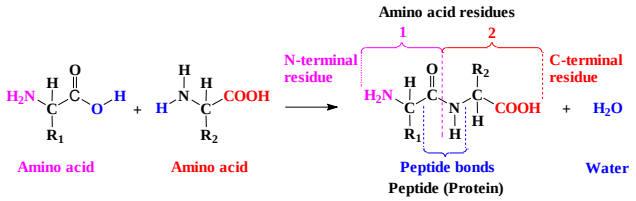

Figure 6. Protein synthesis from amino acids

The results of the above study indicate that the higher the concentration of ethanol extract of Dengen leaves, the higher the percentage of free radical inhibition of DPPH. Similarly, the higher the concentration of ethanol extract of Dengen leaves, the higher the mortality percentage of *Artemia salina* Leach shrimp larvae. This is reinforced by research data showing that the ethanol extract of Dengen leaves contains bioactive compounds that can inhibit DPPH free radicals and kill *Artemia salina* Leach shrimp larvae. Therefore, the ethanol extract of Dengen leaves has been shown to act as an antioxidant compound and is toxic to *Artemia salina* Leach shrimp larvae.

CONCLUSION

The ethanol extract of Dengen leaves (*Dillenia serrata* Thumb) obtained from the MAE method contains secondary metabolites of alkaloids, flavonoids, saponins, polyphenols, terpenoids, and steroids. The results of the antioxidant activity test of the ethanol extract of Dengen leaves (*Dillenia serrata* Thumb) using the DPPH method showed the value of IC_{50} = 100.363 ppm (strong antioxidant). The results of the toxicity test of the ethanol extract of Dengen leaves (*Dillenia serrata* Thumb) using the BSLT method showed the value of LC_{50} = 18.3443 ppm (very toxic). The results of this study indicate that the ethanol extract of Dengen leaves (*Dillenia serrata* Thumb) has potential as a raw material for anticancer

drugs. Therefore, this research needs to be continued until the isolation and structural elucidation stage so that we can find out secondary metabolites from the ethanol extract of Dengen leaves (*Dillenia serrata* Thumb) which have anticancer activity.

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