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PHYTOCHEMICAL SCREENING, ANTIOXIDANT, ANTIDIABETIC ACTIVITY, AND TOXICITY OF *MELASTOMA MALABATHRICUM* L. LEAVES

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Abstract: Extraction and fractionation have been carried out to determine the content of compounds in Harendong (*Melastoma malabathricum* L.) leaves, antioxidant test using the *1,1-diphenyl-2-picrylhydrazil* (DPPH) method, antidiabetic using α-glucosidase enzyme, and toxicity using the *Bhrine Lethality Test Shrimp* method (BSLT). The study showed that the methanol extract and the ethyl acetate fraction of *M. malabathricum* leaves had secondary metabolites: flavonoids, tannins, and saponins. In contrast, the butanol fraction contained flavonoids and saponins, and the water fraction contained tannins. The hexane fraction of *M. malabathricum* leaves did not contain secondary metabolites. The result of antioxidant and antidiabetic activity tests showed that ethyl acetate fraction has higher activity with IC50 values of 23.08 µg/mL and 3.835 µg/mL. Meanwhile, the BSLT toxicity test of methanol extract and all fractions of *M. malabathricum* leaves against *Artemia salina* L. larva had an LC50 > 1000 g/mL.

Keywords: *Artemia salina,* BSLT, DPPH*, M. malabathricum* L., *α- Glukosidase*

Abstrak: Telah dilakukan ekstraksi dan fraksinasi untuk mengetahui kandungan senyawa yang terdapat dalam daun harendong (*M. malabathricum* L.), serta uji antioksidan menggunakan metode *1,1-difenil-2-picrylhydrazil* (DPPH), antidiabetik menggunakan enzim *α-glukosidase* dan toksisitas dengan metoda *Bhrine Shrimp Letahality Test* (BSLT). Hasil penelitian menunjukkan bahwa ekstrak metanol dan fraksi etil asetat daun *M. malabathricum* L. memiliki senyawa metabolit sekunder yaitu flavonoid, tanin dan saponin, sedangkan fraksi butanol mengandung senyawa metabolit sekunder berupa flavonoid dan saponin serta fraksi air mengandung senyawa metabolit sekunder tanin. Fraksi heksana daun *M. malabathricum* L. tidak mengandung senyawa metabolit sekunder. Pada uji aktivitas antioksidan dan antidiabet, fraksi etil asetat menunujukkan aktivitas yang paling tinggi dengan nilai IC_{50} 23.08 µg/mL dan 3.835 µg/mL. Sedangkan pada uji toksisitas BSLT ekstrak metanol dan semua fraksi daun *M. Malabathricum* L. terhadap larva *Artemia salina* L. memiliki nilai LC_{50}> 1000 µg / mL.

Kata kunci: *Artemia salina,* BSLT, DPPH, *Melastoma malabathricum* L., *α- Glukosidase*

INTRODUCTION

Indonesia is famous for its natural wealth which has various types of plants that are efficacious as medicine. Traditional medicine that has been known and used for generations by the Indonesian. Harendong (*Melastoma malabathricum* L.) is one of the plants used as traditional medicine (Joffry *et al.*, 2012). Indonesian people use this plant for the treatment of various diseases, as antipyretic, analgesic, antidiuretic antileukorea, and can treat various of wounds (Dalimartha, 2000)

Some previous studies of the M. *malabathricum* plant, are the ethanol extract of *M. malabathricum* L. leaves have significant antidiabetic activity and anti hyperlipidemic activity in diabetic rats (Balamurugan, Nishanthini and Mohan, 2014). The acetone extract of *M. candidum* has good bactericidal effect, and broad antibacterial activity in the pH range of 5-8 (Wang, Hsu and Liao, 2008) (Wang, Hsu and Liao, 2008). Methanol extract of *M. malabathricum* L. leaves also has antiproliferative activity that can fight various types of cancer cells depending on its high antioxidant and polyphenolic properties (Zakaria *et al.*, 2011). Methanol extract of *M. Malabathricum* leaves showed significant anticancer activity against MCF-7 cells with IC_{50} value of 7.14 g/mL (Roslen *et al.*, 2014)

The potential of a plant is declared as a medicinal plant because of the presence of bioactive compounds or secondary metabolites from that plant. These bioactive compounds are alkaloids, flavonoids, glycerides, steroids and terpenoids (Bernhoft, 2008). The effectiveness of the components of these active compounds as medicinal plants can be determined through preliminary analysis, including antioxidant, antidiabetic and toxicity tests.

Antioxidants are substances that can inhibit the oxidation process, its protect cells from the dangers of free radicals from the metabolism and other external factors. Chemically, natural antioxidants from plants and foods mainly come from phenol-derived compounds such as flavonoids (quercetin), hydroxyamic acid derivatives, coumarins, vitamin E, organic acids (gallic acid) and vitamin C (ascorbic acid). (Xu *et al.*, 2017). According to (Prakash, Rigelhof and Miller, 2001), the mechanism of antioxidant compounds in preventing disease is by capturing free radicals in the body by donating one or more electrons to free radicals, so that free

radical reactions can be inhibited (Fig 1). Source of antioxidant compounds from plants are in flowers, leaves and fruit. Plants containing bioactive compounds such as flavonoids, alkaloids, and terpenoids are potential raw materials can be used as natural antioxidants (Purwanto, Bahri and Ridhay, 2017)

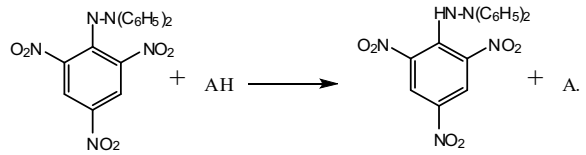

Fig 1. Antioxidants and DPPH Radicals reaction (Kang, Lung and Destiani, 2017)

Antidiabetic is the activity provided by compounds that can treat diabetes. Analysis of antidiabetic activity is usually from herbal plants. The antidiabetic activity was tested in three ways, invitro, invivo, and insilico (Guzmán-Ávila *et al.*, 2018). One of the invitro antidiabetic methods is the *α-glucosidase* enzyme method. The antidiabetic activity of the *α-glucosidase* enzyme method was carried out based on the inhibition of the activity *α-glucosidase* enzyme. The *α-glucosidase* enzyme a role in converting carbohydrates into glucose, therefore if there is inhibition of the activity of α-glucosidase it will lower blood sugar (Bösenberg and Van Zyl, 2008). The analysis was done by adding dimethyl sulfoxide to the sample and adding *p-nitrophenyl α-D-glucopyranoside.* the enzymatic reaction occurred and incubated, the reaction stopped with added Na_2CO_3 and measured absorbance at wavelength of 400 nm. Inhibition occurs when the extract binds to the α-glucosidase enzyme, the ability of the enzyme to hydrolyze reducing p-NPG (p-nitrophenyl α-D-glucopyranoside) to yellow p-nitrophenol will be inhibited. The absorbance was measured based on the amount of p-nirophenol formed. Measurements done with a spectrophotometer at a wavelength of 400 nm. The less p-nitrophenol formed, the smaller the absorbance value and the higher the inhibitory activity, meaning the higher the antidiabetic activity (Nasution, Rahmah and Abdifi, 2013).

The method to analyze the toxicity is the *Brine Shrimp Lethality Test* (BSLT). The principle of this method is based on the mortality rate of *Artemia salina* Leach shrimp larvae to the extract sample. *Brine Shrimp Lethality Test* (BSLT) is a method commonly used in acute toxicity test because compounds have certain bioactivity are often toxic to shrimp larvae (Kristanti, 2008). The obtained result were calculated as the LC_{50} value (average lethal concentration), the optimum concentration of extract that was able to kill 50% of the population of *A. salina*

larvae. The lower the LC_{50} value, the higher the cytotoxicity effect (Nerdy, 2021). The advantages of this method are fast, easy, the results can be repeated, and unexpensive costs (R. Hamidi, Jovanova and Kadifkova Panovska, 2014).

Antioxidant, antidiabetic and toxicity activities based on phytochemical screening of a natural product are important data in tracking the bioactivity of a plant. This study reveals the phytochemical content of secondary metabolites found in harendong leaves and has biological activity. The activities carried out in this study were antioxidant, antidiabetic and toxicity on *M. malabatricum* leaves. There has been no previous research that revealed the screening of phytochemicals in M. malabathricum leaves and their antioxidant, antidiabetic and toxicity activities.

METHODS

Preparation of Simplicia

Harendong leaves (*M. malabathricum* L.) samples were dried in an oven at 50°C for 48 hours, then ground using a grinder to powder form.

Extraction and fractionation of Simplicia

Simplicia of *M. malabathricum* leaves were extracted by maceration method using 96% methanol. Methanol extract of *M. malabathricum* L. leaves was fractionated liquid-liquid with hexane, ethyl acetate and butanol.

Phytochemical Screening

The phytochemical screening carried out was the content of alkaloids, terpenoids, saponins, tannins, and flavonoids. The phytochemical content of *M. malabathricum* extract was analyzed qualitatively according to the standard method as follows (Praptiwi *et al.*, 2020)

Antioxidant

The antioxidant activity was evaluated according to Yen et al., with slight modification (Megawati *et al.*, 2017).

Antidiabetic

The enzyme inhibition activity for *α-glucosidase* (Artanti *et al.*, 2019) was assessed according to the methods reported by Kim et al, with minor modifications.

Toxicity (Budaraga and Putra, 2021)

Shrimp larvae are hatched in dark and bright vessels (Budaraga and Putra, 2021). In the vessel filled with ± 50-100 mg of shrimp eggs to be hatched, then the vessel is divided into 2 parts of the dark zone and the light zone which is given a lamp that is turned on for 48 hours. The dark zone is where the larvae egg are, while the bright

zone is placed with lights to provide lighting in the hatching and separating between larvae. 4 mg of methanol extract of *M. malabathricum* L. leaves and the fraction were dissolved in 10 µg/mL of *Dimethyl Sulfoxide* (DMSO) and 2 mL of seawater (final concentration 2000 µg/mL). Then made dilution concentrations of 1000, 400, and 40 µg/mL. 10 - 11 Artemia salina larvae in 100 µL of seawater were put into well plate 96 and then 100 µL of extract solution was added for each concentration. For each concentration, three repetitions were carried out (triplo). After 24 hours, dead larvae were counted to determine the LC_{50} value using linear regression analysis. LC_{50} is the concentration of the extract that causes 50% of larva mortality.

Statistical Analysis

Data were analyzed using Microsoft Excel and reported as mean ± standar deviation of triplicated determination.

RESULT AND DISCUSSION

Extraction and Fractination of M. malabathricum (M. malabathricum L.) Leaves

The extraction method used is the maceration process. The maceration process was chosen because its a simple method that is carried out by immersing simplicia powder in a suitable solvent, for a period of time. The solvent used is methanol. Methanol is a universal solvent because it can attract all compounds from polar to non-polar compounds. The results of repeated maceration were combined and evaporated. the results were 36 grams of methanol extract, with extract yield of 7.2% (w/w). the result of fractionation from methanol extract of *M. malabathricum* with n-hexane, ethyl acetate, n-butanol and water are 17.62; 27.1; 13.43 and 40.73% respectively, for weight of the methanol extract as 36 grams.

Phytochemical Screening of Methanol Extract and Fraction of M.malabathricum L. Leaves

Phytochemical screening was carried out to identify of bioactive compounds in plants. The results of phytochemical screening on the methanol extract and the fractions of hexane, ethyl acetate, butanol and water of M. malabathricum leaves was showed in Table 1. Base on Result of the phytochemical screening, it can be seen that the methanol extract of M. malabathricum leaves contains secondary metabolites are flavonoids, tannins, and saponins.

Table 1. Chemical Content of Methanol Extract and Fraction of M. malabathricum (*M malabathricum* L.) Leaves

No	Test	Me-OH Extract	F1	F2	F3	F4
1	Flavonoid	+	-	++	+	-
2	Tanin	+	-	++	-	+
3	Terpenoid	-	-	-	-	
4	Saponin	++	-	++	+++	-
5	Alkaloid					
	a. Meyer	-	-	-	-	-
	b. Dragendorf	+	+	+	+	+
	c. Bouchardat	-	-	-	-	-

Note: F1: Hexan Fraction; F2: Etil asetat Fraction; F3: Butanol Fraction; F4:Water Fraction

(-) : not detected

(+) : detected

(++) : strong detected

(+++) : very strong detected

The ethyl acetate fraction contains the same bioactive compounds as the methanol extract, with a higher quantity than the extract, this can be seen from the intensity of the color formed when the extract and fraction are reacted with reagents. The butanol fraction only contains flavonoids and saponins, while the water fraction only contains tannins.

Antioxidant

The antioxidant method used in this study was the DPPH method. Its simple method which developed to determine the antioxidant activity of a substance/extract using the DPPH radical (*2,2-diphenyl -1-pichrylhydrazyl*). DPPH radical is an organic compound containing unstable nitrogen with strong absorbance at a wavelength of 515-517 nm and dark purple color. After reacting with antioxidant compounds, the DPPH will be reduced and the color will change to yellow. The greater the ability to inhibit DPPH free radicals, the greater the ability of antioxidant activity. The inhibition of extract against free radicals was plotted with the concentration of extract to produce a graph of y=bx+a. from graph, value of IC_{50} can be calculated. The IC_{50} value is sample concentration that can inhibit free radicals by 50%. The smaller the IC_{50} value, the higher the inhibitory activity against free radicals (Molyneux P, 2004). The lower the concentration of the sample for inhibit radical, it indicates that the sample has high antioxidants. In this case, it is expected that free radicals can be inhibit by antioxidant compounds with low concentrations. IC_{50} values of Vitamin C, methanol extract and fraction of *M. malabathricum* leaves was showed in table 2 and table 3.

Table 2. Inhibition concentration (IC_{50}) of Vit C.

Sample	Inhibition of DPPH (%) at concentration (µg/mL)				IC_{50} (µg/mL)
	1	5	10	20	
Vit C	5.086±0.424	34.357±1.501	69.143 ±0.008	93.730 ±0.424	8.83±0.111

Table 3. Inhibition concentration (IC_{50}) Methanol Extract dan Fractions of M. malabathricum (*M.malabathricum* L.) Leaves

Sampel	Inhibition of DPPH (%) at concentration (µg/mL)				IC_{50} (µg/mL)
	10	25	50	100	
Me-OH extract	3.38±0.4	16.50±0.8	38.01±0.4	68.14±0.1	64.84±0.1
F1	0.01±0.7	1.45±0.1	9.17±0.4	13.97±0.4	318.71±0.1
F2	17.10±2.1	55.18±0.5	85.04±0.4	92.98±0.4	23.08±0.1
F3	5.80±0.5	21.29±2.1	40.82±1.3	66.42±3.1	71.34±0.4
F4	0.04±0.1	0.18±0.5	0.27±0.4	5.73±0.4	791.52±0.3

Note: : F1: Hexan Fraction; F2: Etil asetat Fraction; F3: Butanol Fraction; F4:Water Fraction

Based on the data above, the ethyl acetate fraction of M. *malabathricum* leaves has the lowest IC_{50} (23.08 g/mL) among all fractions. The ethyl acetate fraction has the highest free radical inhibition compared to other fractions and methanol extracts. this is influenced by the content of chemical compounds contained in the ethyl acetate fraction. Compounds content in the ethyl acetate fraction contain many flavonoid compounds with OH groups that can ward off free radicals. While the butanol fraction has inhibition of 71.34 µg/mL, which is greater than the ethyl acetate fraction, this is possible in the butanol fraction only has a few compounds containing OH groups. The hexane and water fractions have a fairly high IC_{50} , are 318.708 µg/mL and 791,52 µg/mL, possibly the hexane and water fractions not contain phenolic compounds, the hexane and water fractions have low antioxidant activity. Phenolic are one of the antioxidant compounds that have aromatic and hydroxy groups. Its has the ability to scavenge free radicals(Shahidi and Naczk, 1995). phenolic acids, tannins and flavonoids are phenolic group compounds. Therefore, methanol extract. ethyl acetate, and butanol fraction has the ability to scavenge free radicals.

The antioxidant activity of the methanol extract, ethyl acetate, and butanol fraction was related to the content of secondary metabolites contained therein. In table 1 can be seen that the phytochemical content contained in the methanol extract, ethyl acetate and

butanol fraction contains the same chemical compounds, are flavonoids, tannins and saponins. The ethyl acetate fraction had the highest activity among all as seen from the quality of the color changes formed in the phytochemical flavonoid test, meaning that the flavonoid content of the ethyl acetate fraction was high and affected antioxidant activity (Tomsone and Kruma, 2013) stated that the difference in free radical scavenging activity of DPPH depends on the use of the solvent and the content of the extract. Semi-polar solvents such as ethyl acetate have strong abilities as free radical scavengers DPPH methode compare to hexan, buthanol and chloroform (Hartati, Nadifan and Fidrianny, 2020).

Antidiabetic

IC_{50} of Extract and fraction of M. malabathricum leaves was showed in figure 2.

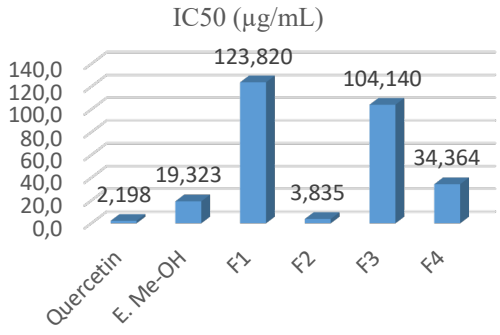

Fig 2. IC_{50} of Methanol Extract and Fraction of M. malabathricum (*M.malabathricum* L.) Leaves. Noted F1: Hexan Fraction; F2: Ethyl acetat Fraction; F3: Buthanol Fraction; F4: Water Fraction

Based on the *α-glucosidase* enzyme inhibition test, the ethyl acetate fraction had the highest inhibitory activity compared to methanol extract and other fractions. It can be seen from its IC_{50} value that the ethyl acetate fraction has the smallest IC_{50} of 3.835 µg/mL, which is Approaching IC_{50} of quercetin standard meaning that at concentration of 3.835 µg/mL the ethyl acetate fraction was able to inhibit the *α-glucosidase* enzyme.

The inhibitory activity of the *α-glucosidase* enzyme was influenced by chemical content in the sample. Based on the phytochemical results of methanol extract and fraction *M. malabathricum* leaves, the ethyl acetate fraction contains flavonoids, tannins and saponins. The flavonoid compounds contained in the ethyl acetate fraction are thought to be inhibitors of the *α-glucosidase* enzyme. According to (Xu, 2010) The hydroxyl group (-OH) contained in flavonoids has an important role in the inhibition of *α-glucosidase* enzymes, especially in the C ring of flavonoids (Fig 3).

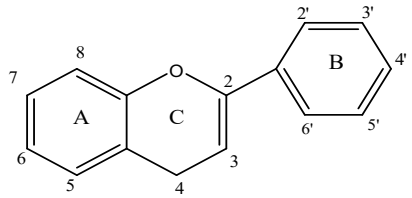

Fig 3. Flavonoid StructureResearch conducted by (Yilmazer-Musa *et al.*, 2012)

Reported that the presence of gallat

group (-$C_7O_4H_5$) at position 3 in the C ring of flavonoids would interact with the *α-glucosidase* enzyme so that the activity of the enzyme was inhibited.(Tadera *et al.*, 2006), adding the presence of a hydroxyl group on the substitution ring B of flavonoids are known to increase the inhibitory activity of the *α-glucosidase* enzyme. Other mechanism of flavonoids (quercetin) as antidiabetic is by inhibiting intestinal GLUT 2 so that it can reduce glucose absorption. This causes the reduction of glucose and fructose from the intestines so that blood glucose levels decrease. GLUT 2 is considered a major glucose transporter under normal conditions. In a Song study , it was found that flavonoids can inhibit glucose. When quercetin is ingested with glucose, hyperglycemia is significantly decreased. This shows that quercetin can inhibit glucose absorption through GLUT 2 (Ajie, 2015) .

Table 4. Toxicity of Methanol Extract and Fraction of M. malabathricum (*M.malabathricum* L.) Leaves

No	Extract	Kons(µg/mL)	Mortality	LC_{50} (µg/mL)
1	Methanol Ex	1000	43.590	>1000
		500	16.071	
		200	5.128	
		20	0.962	
2	F1	1000	45.000	>1000
		500	17.857	
		200	5.263	
		20	0.000	
3	F2	1000	48.837	>1000
		500	22.414	
		200	8.000	
		20	0.000	
4	F3	1000	44.737	>1000
		500	15.094	
		200	2.740	
		20	0.000	
5	F4	1000	37.838	>1000
		500	12.727	
		200	2.564	
		20	0.000	

Information : F1: Hexan Fraction; F2: Etil asetat Fraction; F3: Butanol Fraction; F4:Water Fraction

Toxicity Analyze

The BSLT toxicity analyze was carried out by determining the LC_{50} value of the components extract of natural product against *Artemia salina* L. larvae. Based on the BSLT method, an extract was stated toxic if it cause the death of 50% of the test animals at a concentration of less than 1000 µg/mL. Observations were made 1x24 hours after the sample was inserted into the well containing 10 larvae and was repeated 3 times to get significant results.

Based on Table 4, the sample LC_{50} was determined by linear regression analysis. The LC_{50} value was obtained from the calculation between the log concentration and % mortality of *A.salina* L. larvae. From Table 4 it can be seen, Mortality methanol extract and fractions of *M. malabathricum* leaves has a small value, indicating that few died larvae both at low and high concentrations. It can be stated that the methanol extract and all fractions did not contain chemical compounds that have potential toxicity by showing a relatively large LC_{50} value its more than 1.000 µg/mL.

CONCLUSION

The methanol extract and the ethyl acetate fraction of *M. malabathricum* leaves have secondary metabolites of flavonoids, tannins and saponins, the butanol fraction contains flavonoids and saponins and the water fraction contains tannins. The antioxidant activity of methanol extract has IC_{50} value of 64.840 µg/mL, while the ethyl acetate fraction is able to inhibit free radicals IC_{50} value of 23.080, and butanol 71.340 µg/mL. the antidiabetic test showed the best results, its the ethyl acetate fraction with an IC_{50} value of 3.835µg/mL. In the BSLT toxicity test against *A.salina* larvae, methanol extract and all fraction of *M. malabathricum* leaves had LC_{50} values > 1000 µg/mL. This indicates that the extract and all fraction of *M. malabathricum* leaves non toxic.

The data's obtained at this study, it is necessary to carry out further research to get flavonoids, saponins and tannins type contained in the ethyl acetate fraction of M.malabatricum leaves. The ethyl acetate fraction had the best activity among all fraction and the methanol extracts.

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