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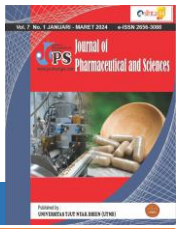

In Vitro Antidiabetes Activity Test of 96% Ethanol Extract from *Melastoma malabathricum* L. Leaves Using Maceration Method

Uji Aktivitas Antidiabetes *In Vitro* Ekstrak Etanol 96% Daun Karamunting (*Melastoma malabathricum* L.) dengan Metode Maserasi

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Abstract

Background: Diabetes mellitus remains a major global health issue, with rising prevalence due to population growth, aging, and lifestyle factors. *Melastoma malabthricum* L. has potential as an antidiabetic agent based on empirical data and in vivo study. **Objective**: To determine the content of secondary metabolite compounds contained in the extract of Karamunting leaves and to determine the activity and EC_{50} value of the extract for antidiabetes was evaluated using the Nelson-Somogyi method. **Methods:** This study used the phytochemical screening approach to identify the active chemicals. 96% ethanol was the solvent used in the maceration process to extract the leaves of M. malabathricum. The Nelson-Somogyi method was used to test the antidiabetic activity by calculated the sample's EC_{50} value on decreased glucose levels. The Nelson Somogyi method's antidiabetic activity was tested with a UV-Vis Spectrophotometer. It operated for 25 minutes at a wavelength of 740 nm. **Results:** Phenolic chemicals, flavonoids, saponins, steroids, and tannins all exhibited positive results from the phytochemical screening. After the addition of 96% ethanol extract of M. malabathricum leaves at gradually higher concentrations, the percentage decrease in glucose levels was 25.32%, 36.90%, 51.39%, 70.87, and 82.83% at concentrations of 1, 2, 3, 4, and 5 ppm. R2 = 0.9927 with y = 14.898x + 8.7747. **Conclusion:** 96% ethanol extract of M.malabathricum leaves contains several active substances, can reduce blood glucose levels and has an EC_{50} value of 2.76 ppm. Clinically, this implies a promising therapeutic potential with lower required dosages.

***Keywords*: Diabetes Mellitus, Glucose Levels, Nelson-Somogyi, EC_{50}.**

Abstrak

Latar Belakang: Diabetes melitus masih menjadi masalah kesehatan global yang utama, dengan prevalensi yang terus meningkat akibat pertumbuhan populasi, penuaan, dan faktor gaya hidup. *Melastoma malabthricum* L. berpotensi sebagai agen antidiabetik berdasarkan data empiris dan studi in vivo. **Tujuan:** Mengetahui kandungan senyawa metabolit sekunder yang terdapat pada ekstrak daun Karamunting dan mengetahui aktivitas dan nilai EC_{50} dari ekstrak terhadap antidiabetes dengan metode *Nelson-Somogyi*. **Metode:** Penelitian ini menggunakan pendekatan skrining fitokimia untuk mengidentifikasi bahan kimia aktif. Etanol 96% adalah pelarut yang digunakan dalam proses maserasi untuk mengekstrak daun *M. malabathricum*. Metode Nelson-Somogyi digunakan untuk menguji aktivitas antidiabetik dengan menghitung nilai EC_{50} sampel pada penurunan kadar glukosa. Aktivitas antidiabetik metode Nelson Somogyi diuji dengan Spektrofotometer UV-Vis. Alat ini dioperasikan selama 25 menit pada panjang gelombang 740 nm. **Hasil:** Senyawa fenol, flavonoid, saponin, steroid, dan tanin semuanya menunjukkan hasil positif dari skrining fitokimia. Setelah penambahan

ekstrak etanol 96% daun *M. malabathricum* dengan konsentrasi yang semakin tinggi, persentase penurunan kadar glukosa adalah 25,32%, 36,90%, 51,39%, 70,87%, dan 82,83% pada konsentrasi 1, 2, 3, 4, dan 5 ppm. R^2 = 0,9927 dengan y = 14,898x + 8,7747. **Kesimpulan:** Ekstrak etanol 96% daun M. malabathricum mengandung beberapa zat aktif, dapat menurunkan kadar glukosa darah dan memiliki nilai EC_{50} sebesar 2,76 ppm. Secara klinis, hal ini menyiratkan potensi terapi yang menjanjikan dengan dosis yang dibutuhkan lebih rendah.

Kata Kunci: Diabetes Melitus, Kadar Glukosa, Nelson-Somogyi, EC_{50}.

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Introduction

Indonesia ranks fifth globally in the prevalence of Diabetes Mellitus, following China, India, the United States, and Pakistan. According to the International Diabetes Federation [1], approximately 537 million people worldwide suffer from diabetes, resulting in 6.7 million deaths annually. In Indonesia, diabetes affects 19.4 million individuals aged 20-79, with a mortality rate of 236,000 people. Management of diabetes can be achieved through non-pharmacological and pharmacological interventions. Non-pharmacological approaches involve lifestyle modifications, such as weight loss, increased physical activity, and smoking cessation [2]. Meanwhile, pharmacological therapy utilizing natural products as an alternative treatment modality shows promise in minimizing adverse side effects [3].

Indonesia has abundant natural resources that are effective as medicines, one of which is *Melastoma malabathricum* L. This plant commonly known as Karamunting or Senduduk, is a plant species from the Melastomataceae family native to South Kalimantan. This plant has been traditionally utilized for its medicinal properties, particularly its leaves, which are empirically used to treat diabetes, hypertension, and infections [4]. Phytochemical analysis of the 96% ethanol extract of *M. malabathricum* leaves revealed the presence of glycosides, flavonoids, tannins, steroids, and saponins [5]. The antidiabetic potential of *M. malabathricum* L. is attributed to the synergistic effects of its chemical compounds, particularly flavonoids, which have been shown to reduce glucose levels by inhibiting enzyme activity, enhancing insulin secretion, reducing apoptosis, and mitigating insulin resistance [6].

In vivo studies conducted showed antidiabetic activity of 96% ethanol extract of *M. malabathricum* L. in alloxan-induced male mice [7]. Administration of the extract at doses of 100, 200, and 400 mg/kg body weight resulted in significant reductions in blood glucose levels, with values of 97.3 ± 2.44, 86.8 ± 2.15, and 86.3 ± 5.52 mg/dL, respectively, after 15 days of treatment. Furthermore, a study evaluated the antidiabetic activity of the same extract using the α-glucosidase inhibition method [4]. The results showed varying IC_{50} values for extracts obtained through maceration (879.559 µg/mL) and reflux (1061.631 µg/mL) techniques, compared to the positive control acarbose (0.255 µg/mL). Notably, the maceration technique yielded higher inhibitory activity against α-glucosidase, likely due to the preservation of heat-sensitive phytochemical compounds that are crucial for enzyme inhibition.

To date, no studies have investigated the antidiabetic activity of 96% ethanol extract of *M. malabathricum* leaves using the Nelson-Somogyi method. This method was chosen in the antidiabetic test because it can measure the concentration of reducing sugars which are a direct indicator of the activity of carbohydrate-digesting enzymes, so that the inhibitory effect of the compounds contained in the sample can be assessed [8]. This study aims to evaluate the antidiabetic potential of the extract in vitro. The objective is to determine

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whether *M. malabathricum* leaves exhibit efficacy in reducing glucose levels, thereby contributing to the understanding of its potential therapeutic applications.

Experimental Section

Materials and Apparatus

The materials used are aquadest, amyl alcohol, anhydrous acetic acid, dragendorff reagent, ethanol 96%, hydrochloric acid, iron (III) chloride, mayer reagent, magnesium powder, glucose, liebermann burchard reagent, arsenomolybdate reagent, nelson-Somogyi reagent, and wagner reagent.

The apparatus used are analytical balance (Fujitsu®), set of maceration tools, rotary evaporator (IKRF10®), waterbath (Memmert®), micropipette (TopPette Pipettor), set of UV-Vis spectrophotometer (PG Instrument®), and various glassware (Iwaki, Pyrex).

Determination

The plant was collected from Mataraman District, Banjar Regency, South Kalimantan Province, Indonesia. The plant was identified at the Laboratory of Fakultas MIPA Universitas Lambung Mangkurat Banjarbaru with number 297a/LB.LABDASAR/XII/2023 and the results showed sample namely *M. malabathricum* L. from the Melastomataceae family.

Figure 1. *M. malabathricum* (a) Plant (b) Leaves

Preparation of Simplicia

The sample used was *M. malabathricum* leaves which were still fresh and green, neither too old nor too young. The method begins with collected 3.5 kg of ingredients, wet sorted, washed, chopped, dried, dry sorted, pollination, storage and then finally container [9]. Simplicia powder was made to a fine degree of coarse powder used mesh no. 20.

Extraction

The leaves of *M. malabathricum* were powdered and subjected to maceration in 96% ethanol solution (1:7 ratio) at room temperature, protected from sunlight. The mixture was stirred periodically for 6 hours, followed by an 18-hour resting period, and this process was repeated twice. The resulting filtrate was collected and concentrated using a rotary evaporator at 50°C with 40 rpm and waterbath at 50°C until a constant weight was achieved [10, 11]. The percentage yield of the crude extract was then calculated from the resulting thick extract [12]. The following equation can be used to determine the extract yield:

$$\% \text{ extract yield} = \frac{extract\ weight}{simplicia\ weight}\ x\ 100\% \quad \ldots\ldots\ldots\ldots\ldots\ (1)$$

Phytochemical Screening

To carried out phytochemical screening, first a sample solution was made in a ratio of (1: 1). The method was to dissolve 10 mg of thick extract with 10 mL of 96% ethanol in a glass beaker and then identified it [13].

Alkaloids

A test tube contained one milliliter of the sample solution was filled by added a milliliter of hydrochloric acid 2 N. After two minutes of heated over a waterbath, it was chilled. In the first test tube, the extract sample

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was then filtered and 3-5 drops of Dragendorff's reagent were added. A red precipitate will form if it is positive for alkaloids. In the second tube, the extract sample was put into a test tube and then 2 drops of Mayer's reagent were added, a white precipitate would form. In the third tube, the extract sample is put into a test tube and then added with 2 drops of Wagner's reagent and a brown or brownish orange precipitate will form [14].

Flavonoids

One mL of the sample solution was taken, then 0.2 g of magnesium was added and dripped with concentrated HCl and amyl alcohol in a test tube. A positive result for flavonoid compounds if the color changes from yellow to red [15].

Phenols

A test tube was filled with one mL of the sample solution and one mL of 1% $FeCl_3$. The existence of phenolic chemicals can be determined by a precipitate that is blackish green [16].

Saponins

Two mL sample solution was shaken with 2 mL of hot water. If the white foam that appears lasts for 10 minutes, it indicates a positive result for saponin compounds [13].

Steroids/Triterpenoids

The sample solution was taken as much as 1 mL, then Liebermann-Burchard reagent was added (anhydrous CH_3COOH: concentrated H_2SO_4). The presence of steroids is indicated by a blue or green color, while triterpenoids give a red or purple color [17].

Tannins

One mL of the sample solution was obtained, and then one milliliter of a 1% gelatin solution with NaCl was added. The existence of tannin compounds can be recognized by white precipitate [18].

Antidiabetic Activity Test

In antidiabetic activity test, the Nelson Somogyi method consists of several stages : preparation of solutions and reagents, maximum wavelength determination, determination of operating time, determination of glucose levels.

In solution preparation, a 1000 ppm glucose stock solution was made which was diluted to a 50 ppm working solution. The 1000 ppm glucose stock solution was made by weighing 0,01 g of anhydrous glucose and then dissolving it with distilled water in a 10 mL measuring flask [19]. Nelson-Somogyi reagent was made by mixed 25 mL of Nelson A solution and 1 mL of Nelson B solution [20]. Blank solution was made by 1 mL of Nelson-Somogyi reagent added 1 mL of distilled water, then covered the test tube with aluminum foil and heated in boiled water for 10 minutes. After cooled the solution for five minutes, the sample was placed into a 10-milliliter measuring flask, and one milliliter of arsenomolybdate reagent was added. It followed by homogenizing and assessed with a UV-Vis spectrophotometer after having been diluted with distilled water to the limit [19].

Maximum wavelength determination was done by took 1 mL of 50 ppm glucose solution into a test tube and added 1 mL of Nelson-Somogyi reagent, then homogenized the solution and then covered the test tube with aluminum foil. Heat the solution for 10 minutes over boiled water and let it cool for 5 minutes, then transfer the solution into a 10 mL measuring flask, add 1 mL of arsenomolybdate reagent to the solution, then dilute with distilled water to the 10 mL mark, homogenized. Determination of the maximum wavelength between 700-780 nm [8]. Determination the operating time had the same procedure as determination the maximum wavelength, but the absorbance of the sample was measured at the wavelength that has been obtained, which was measured for 30 minutes with a 1 minute interval, so that a stable operating time can be produced [19].

Determination of glucose levels for 96% ethanol extract of *M. malabathricum* leaves was made in a series of concentrations of 1, 2, 3, 4, 5 ppm. Five mL of the sample and 5 mL of a 50 ppm glucose solution were added, and the mixture was shaken. After taking 1 mL of the solution combination, 1 mL of Nelson-Somogyi reagent was added, and the mixture was wrapped in aluminum foil. For ten minutes, the solution mixture was heated over boiling water. After cooling for 5 minutes, it was moved into a 10-milliliter measuring flask, where 1 milliliter of the arsenomolybdate reagent was added. It was then diluted with distilled water to the

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appropriate level, shaken, and allowed to run for the duration of the experiment. A UV-Vis spectrophotometer set to its maximum wavelength will be used to read the data, and the percentage of glucose levels will then be determined [21].

Data Analysis

The absorbance that has been obtained and the sample measurements will be compared with the standard glucose solution to determined the percent reduction in glucose levels. Calculation of the percentage of glucose reduction levels uses the following formula:

$$A = \frac{C-B}{C} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots (2)$$

A = % antidiabetic activity
B = residual glucose absorbance
C = positive control absorbance (glucose + Nelson-Somogyi)

The size of the sample in reduced glucose levels can be expressed by the EC_{50} value. Calculated used the linear regression equation formula where the concentration of the sample was expressed as (x) and the result of the percentage reduction in glucose levels was expressed as (y). The regression equation y= bx + a was used to determine the value of EC_{50} with the equation:

$$EC_{50} = \frac{50-a}{b} \quad \ldots\ldots\ldots\ldots\ldots (3)$$

EC_{50} = Value that can provide effectiveness in reducing the level of glucose is 50%
a = intercept
b = slope/value of the slope of the curve

Results and Discussion

The preparation of simplicia begins with the collection of materials carried out in the morning, with the aim of obtaining high active compounds, because if picked during the day the plants will experience photosynthesis so that the active compounds that will be extracted are less than optimal [22]. The collection was also not carried out at night, because at night plants do not photosynthesize, even need oxygen and emit carbon dioxide [23]. The sample was carried out in Pematang Danau Village because some of the areas tend to still be surrounded by forests, this is in accordance with the Karamunting plant which usually lives wild in the forest, and it is known that the existence of the Karamunting plant in Pematang Danau Village is very easy to find and in large quantities.

M. malabathricum leaves were taken and then wet sorted, washed, shredded, dryed, dry sorted, refined and sieved were carried out. Wet sorted aimed to separate impurities, namely external impurities such as soil, gravel that was still attached and parts of the plant that are not used. Washed to remove other impurities that are attached to the simplicia [24]. Then shredded which aimed to facilitate the dryed process so that the dryed time can be saved, besides also facilitated the grinded of the simplicial.

The simplicia was dried in the sun, covered with a black cloth so that it was not exposed to direct sunlight because it could damaged chemical compounds if exposed to UV rays for too long. Dried was carried out in order to prevent the growth of bacteria and fungi during the storage stage. The initial parameter for stopping the dryed process was marked by the *M. malabathricum* leaves that can be crumbled [25]. Then the dry sorted stage aimed to separate unwanted plant parts and other impurities that are still left in the dry simplicial. Furthermore, pollination used a blender aimed to reduce the size of the simplicia particles to be used. The pollination results are then sieved using sieve no. 20. The yield of the karamunting leaf simplicia was obtained at 18.88%. The lower the yield value indicates the lower the water content in the simplicia powder. Likewise, the high water content in the herbal medicine will affect the quality of the herbal medicine [11].

In the extraction, Maceration was chosen as method because it avoids the destruction of heat-sensitive compounds, allowing the extraction of thermolabile phytoconstituents. This method maintains the integrity of the bioactive compounds responsible for glucose-lowering activity. 96% ethanol solvent was chosen because in previous studies *M. malabathricum* leaves were able to reduce blood glucose levels in mice. To further ensure the antidiabetic activity of *M. malabathricum* leaves but in vitro, 96% ethanol solvent was used [7]. The yield value was related to the amount of bioactive content contained in plants, the higher the extract

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yield, the higher the substance content [26]. The yield in this study was quite high, namely 15.885 % compared to another study [27] that used the same extraction method.

The results obtained from phytochemical screening can be seen in Table 1. This stage aimed to determine the secondary metabolite content found in *M. malabathricum* leaves. The compounds that play a role in antidiabetes activity such as flavonoids, phenols, saponins, steroids and tannins.

Table 1. The Phytochemical Screening Results of *M. malabathricum* leaves

Compounds	Positive Results References	Results	Information	Pictures
Alkaloids				
a. Dragendorff	Red orange precipitate	-	No red/orange precipitate formed	
b. Mayer	White precipitate	+	Formation of a white precipitate solution	
c. Wagner	Reddish brown precipitate	-	No reddish brown precipitate is formed.	
Flavonoids	Red or orange on the amyl alcohol layer	+	Formation of red or orange on the amyl alcohol layer	
Phenol	Green, purple, blue or black color	+	Formation of a blackish green solution	
Saponins	A stable foam is formed	+	Foam is formed, does not disappear after adding 2N HCl	
Steroids	Blue or green	+	formation of a bluish green solution	
Trriterpenoids	Red or purple	-	No red/purple solution	
Tanins	Yellowish white precipitate	+	Formation of yellowish white precipitate	

The antidiabetic activity measured by the Nelson–Somogyi assay is mainly attributed to the presence of flavonoids, particularly quercetin and its derivatives, which act as potent inhibitors of carbohydrate-hydrolyzing enzymes [28]. In addition, polyphenols [29], tannins, and saponins [30] may synergistically contribute to reducing glucose release, thereby enhancing the overall hypoglycemic effect of the extract. Previous research on *Melastoma malabathricum* L. showed that flavonoid compounds act as antidiabetics by inhibiting α-amylase and α-glucosidase, thereby slowing the breakdown of starch into glucose [28, 31]. They also act as potent antioxidants by reducing oxidative stress associated with insulin resistance [32]. Furthermore, these compounds can stimulate pancreatic β cells to secrete more insulin [33]. Phenolic compounds can increase insulin secretion, prevent damage to pancreatic β cells and increase the function of pancreatic β cells so that they can cause hypoglycemic effects through their ability as antioxidants that are useful for regenerating cells and counteracting free radicals which are needed for the therapy of degenerative diseases, such as diabetes mellitus [34]. Saponin is also a bioactive compound against diabetes, saponin is able to lower blood sugar levels with a working mechanism as an antihyperglycemia by stimulating the release of insulin in pancreatic [35]. Steroids/Triterpenoids are also one of the compounds that have antidiabetic activity, the mechanism of action of steroids and triterpenoids is by inducing the release of insulin from the pancreas, so that blood glucose levels will decrease. The mechanism of tannin in lowering blood sugar levels is to precipitate intestinal mucous membrane proteins and form a protective layer of the intestine, thereby inhibiting glucose intake [33]. Tannin can trigger the metabolism of glucose and fat so that these two sources of calories in the blood can be avoided. This tannin compound also has hypoglycemic activity, namely by increasing glycogenesis [4].

The glucose level reduction activity test was carried out using a UV-Vis spectrophotometer which was preceded by determining the maximum wavelength and the obtained at 740 nm. The maximum wavelength produced in this study is included in the maximum wavelength range in the literature, namely the range of

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700-780 nm which is the glucose absorption area [36]. Operating time is the time required for a compound to react perfectly with another compound to obtain a stable or constant solution compound. The operating time was produced at 25-27 minutes. The time chosen for this study was 25 minutes. This result is the same as the research conducted by another research [36, 37] which also obtained an operating time at 25 minutes. In decreased the glucose levels of 96% ethanol extract of t *M. malabathricum* leaves the absorbance of 50 ppm glucose working solution was first determined. The decreased in glucose levels after added sample could be seen in table 2 and Figure 2. Table 2 showed that the higher the concentration of *M. malabathricum* leaves extract, the greater the decrease in glucose levels.

Table 2. Results of Percentage Reduction in Glucose Levels

Conc. (ppm)	% decrease in glucose levels (replication to-)			Average ± SD	Linear regression	EC_{50} (ppm)
	I	II	III			
1	25,25	27,59	23,12	25,32 ± 2,23	y = 14,898x + 8,7747	2,76
2	36,87	37,09	36,75	36,90 ± 0,17		
3	50,61	51,39	52,17	51,39 ± 0,78		
4	67,70	71,39	73,51	70,87 ± 2,93		
5	82,56	83,46	82,45	82,83 ± 0,55		

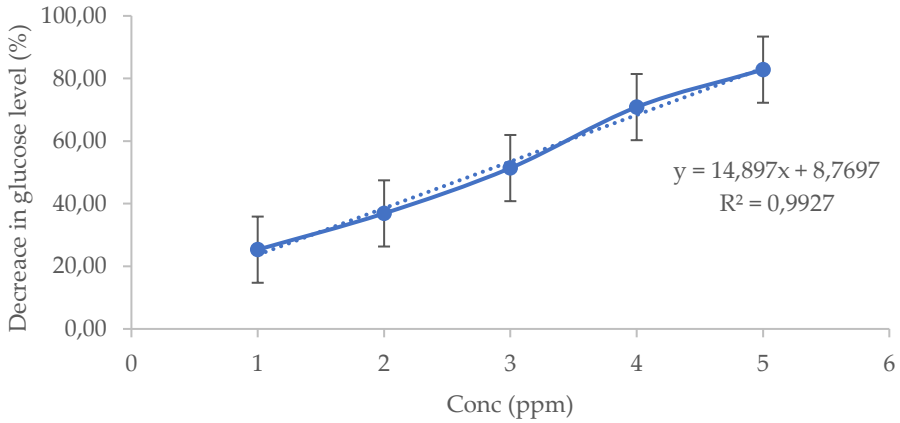

Figure 2. Glucose Decrease Curve

Activity test of glucose reduction of 96% ethanol extract of *M. malabathricum* leaves used Nelson-Somogyi method conducted in vitro. This method was chosen because it was more specific when used in determined reduced sugar levels in samples that already have mixed sugar compounds in them. The addition of Nelson reagent was intended to reduce cupric oxide to cuprous oxide (Cu_2O), where K-Na-tartrate in Nelson reagent functions to prevent the precipitation of cupric oxide. After the addition of Nelson reagent, the solution containing glucose with Nelson reagent was heated for 10 minutes in boiling water, the purpose of heated was to accelerate the reduction process of cupric oxide to cuprous oxide [21].

After ten minutes the solution was removed and then cooled so that the reaction runs stably, because if the solution was heated too long or hot it will affect the solution because there was a possibility of damage or the components evaporate. After the solution was cold, the solution was added with 1 mL of arsenomolybdate reagent. The addition of this arsenomolybdate reagent aimed to be able to react with the cuprous oxide precipitate. When added this arsenomolybdate reagent, cuprous oxide will reduce the arsenomolybdate reagent back to molybdine blue which was greenish blue whose absorbance will be measured used a UV-Vis spectrophotometer with a maximum wavelength that has been obtained, namely 740 nm [36].

The difference in color intensity produced can indicate the amount of reducing sugar in the sample. The greater the residual glucose content contained in the sample solution, the more concentrated the blue color formed. The more concentrated the blue color, the more light is absorbed and the less light is transmitted so that the absorbance value obtained during measurement will be greater. The greater the concentration of the sample, the smaller the absorbance value so that the percentage of glucose reduction that will be given will be greater. At the smallest sample concentration value, it will provide a large absorbance so that it will provide

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the smallest percentage of glucose reduction, this is because there is still a lot of glucose residue that is not bound by flavonoids [38]. The sample size in reducing glucose levels was expressed by the EC_{50} value which was a value that can provide effectiveness in reducing glucose levels, which is 50%. If the smaller the EC_{50} value obtained, the greater the effectiveness of the sample as a glucose reducer [36].

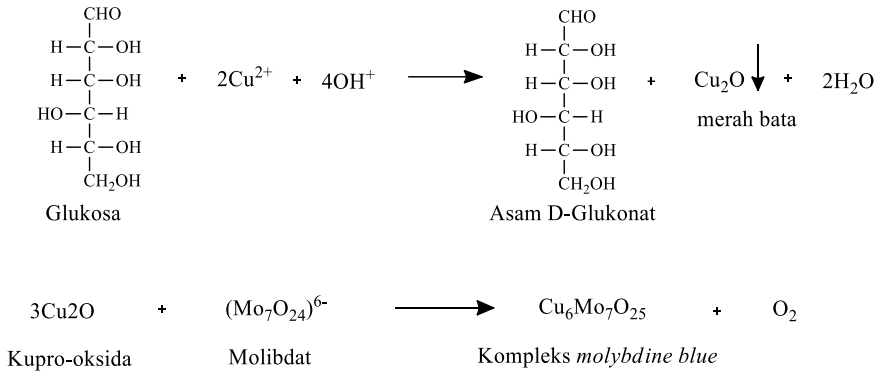

Figure 3. The reaction of the formation of cuprous oxide compounds and molybdine blue complexes

Based on the results of the calculation of the EC_{50}, the sample can reduce glucose levels. The ability of *M.* malabathricum leaves to reduce glucose levels was obtained from the flavonoid compounds identified in extract which have strong free radical scavenging activity that can prevent damage to pancreatic beta cells caused by oxidative stress and can also help increase insulin secretion, and has the ability to stabilize blood sugar by inhibiting the absorption of carbohydrates from food in the intestinal tract [39].

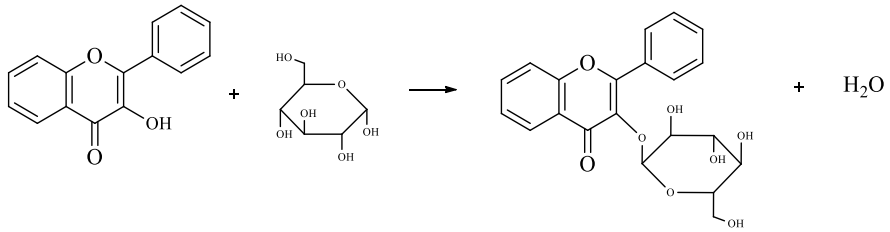

Figure 4. Reaction Between Flavonoids and Glucose

A glucose-flavonoid complex can be created when certain flavonoid molecules combine with glucose. The glucose level in the sample solution will drop as a result of the hydroxyl group in the flavonoids of the sample extracts binding glucose. More flavonoids in the sample extract will bind to glucose at higher concentrations, reducing the amount of glucose in the sample and causing a bigger drop in glucose levels [8].

The results of the in vitro antidiabetic activity test of showed 96% ethanol extract of *M. malabathricum* leaves had very good potential in reduced glucose levels with an EC_{50} value of 2.76 ppm. The EC_{50} value of the tested sample (2.76 ppm) indicated a markedly higher antidiabetic potency compared to previously reported plant extracts such as kale (11.13 ppm) [8] and red lettuce (9.11 ppm) [40], as well as the positive control quercetin (6,831 ppm) [41]. Although slightly less potent than the nanoparticle formulation of parijoto fruit extract (EC_{50} < 2 ppm) [41], the obtained value still demonstrates a strong inhibitory activity, suggesting that the tested sample is a promising natural candidate for antidiabetic therapy. Antidiabetic activity observed in the macerated extract may be attributed to the preservation of thermolabile phytoconstituents, as this method avoids heat-induced degradation. Furthermore, the prolonged solvent–matrix contact during maceration enables more efficient diffusion of bioactive compounds, resulting in a lower EC_{50} value compared to extracts obtained through hot extraction technique. Then accordance to Sahara *et al.* 96% ethanol extract of *M. malabathricum* L. leaves can reduce blood glucose levels in vivo in alloxan-induced male rats [7]. This proves that it can be ascertained that there is activity to reduce glucose levels in 96% ethanol extract of *M. malabathricum* leaves both in vivo and in vitro. Despite its usefulness in determining glucose-reducing activity, the Nelson–Somogyi method has several limitations, including low specificity towards glucose, potential interference from phytochemicals, and reduced sensitivity compared to enzymatic or chromatographic assays. Therefore, the results should be interpreted cautiously and ideally complemented with more specific in vitro or in vivo approaches.

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Conclusions

The results of the study of 96% ethanol extract of *M. malabathricum* leaves contains several active substances, have antidiabetic activity with the Nelson-Somogyi test method an EC_{50} value 2.76 ppm. Clinically, this implies a promising therapeutic potential with lower required dosages.

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