

Phytochemical Screening And Antidiabetic Test Of Ethanol Extract Of Turmeric Leaves (*Curcuma domestica* Val.) On Decreasing Blood Glucose Of Diabetic Rats

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Abstract.

The turmeric plant (*Curcuma domestica* Val.) is a tropical plant that grows a lot on the Asian continent with its use as a food coloring and fragrance. The use of turmeric leaves by residents is only a cooking ingredient not used in large quantities. Even most of these turmeric leaves are considered waste and have minimal utilization. Turmeric leaf methanol extract can be used to lower blood glucose. Turmeric leaf extract has the potential as a valuable food source of its antioxidant components, such as total phenolic compounds and flavonoids which increase radical scavenging activity. In particular, the water extract of turmeric leaves contains high total phenolic compounds (2.741 ± 0.099 mg GAE/g) and flavonoids (4.776 ± 0.010 mg QCE/g). Diabetes mellitus is a metabolic disorder associated with several chronic complications, such as nephropathy, neuropathy, retinopathy and cardiomyopathy. Part of the turmeric plant leaves is reported to have the ability as an antioxidant. Antioxidant compounds can control blood glucose levels and prevent diabetes complications. This study was conducted to test the ability of turmeric leaf ethanol extract (EEDK) to reduce blood glucose levels in diabetic rats induced by nicotinamide and streptozotocin and to perform phytochemical screening to determine the compounds contained in turmeric leaves so that it becomes an alternative as a diabetes treatment and can prevent damage or diabetic complications. The results showed that turmeric leaves contained secondary metabolites, namely flavonoids, glycosides, tannins, and triterpenoids/steroids, but there were no alkaloids or saponins. The characterization results show that the sample meets the requirements. Turmeric leaf ethanol extract doses of 400 mg/kg, 200 mg/kg, and 100 mg/kg could reduce the Blood Glucose Level of NA and STZ-induced rats from the fourth day of treatment.

Keywords: antidiabetic, diabetic, turmeric, *Curcuma domestica* Val., and Characterization.

I. INTRODUCTION

Diabetes mellitus is a metabolic disorder associated with many chronic complications, such as nephropathy, neuropathy, retinopathy and cardiomyopathy. This condition is an accumulation of damage to various organs, including diseases classified as high risk because they can cause death, so they are also called silent killers [1]. The turmeric plant is a tropical plant that grows a lot on the Asian continent with its use as a food coloring and fragrance. The use of turmeric leaves by residents is only a cooking ingredient not used in large quantities. Most turmeric leaves are considered waste and have minimal use [2]. Hasan's research (2014) showed that turmeric leaves' methanol extract can lower blood glucose [3]. According to research by Kim et al. (2019), turmeric leaf extract has the potential to be a valuable food source of its antioxidant components, such as total phenolic compounds and flavonoids which increase radical scavenging activity. In particular, the water extract of turmeric leaves contains high total phenolic compounds (2.741 ± 0.099 mg GAE/g) and flavonoids (4.776 ± 0.010 mg QCE/g) [4]. Based on research conducted by Suryanto, 2019, turmeric leaves have flavonoid bioactive compounds. Flavonoids can capture free radicals and reduce oxidative stress to improve beta cell function [5]. Flavonoids reduce reductase-reductase, regenerate pancreatic cells, increase insulin release and increase Ca²⁺ ion uptake. In addition, flavonoids inhibit the α -glucosidase enzyme, prevent glucose absorption, and improve glucose tolerance [6].

According to research by Yan and Asmah (2010), parts of the leaves of the turmeric plant are reported to have the ability as antioxidants. Antioxidant compounds can control blood glucose levels and prevent diabetes complications [7, 8]. Flavonoids can reduce blood glucose levels by inhibiting gluconeogenesis by increasing the AMP: ATP ratio, activating AMPK, and increasing glucose uptake in skeletal muscle and adipose tissue [9]. Flavonoids also act as antioxidants by donating hydrogen ions and increasing SOD gene expression [10]. Flavonoids can also inhibit the formation of AGEs by trapping

reactive dicarbonyl compounds such as methylglyoxal and glyoxal [11]. Based on the above background, this study was conducted to test the ability of turmeric leaf ethanol extract (EEDK) to reduce blood glucose levels in diabetic rats induced by nicotinamide and streptozotocin and to perform phytochemical screening to determine the compounds contained in turmeric leaves so that they become an alternative as a diabetes treatment and can prevent the damage or complications of diabetes.

II. METHODS

Tools and Materials

The tools used in this study were maceration equipment, a rotary evaporator, glassware, a blender, an electric balance, simplicia drying cupboard, a porcelain cup, desiccator, a set of water content determination tools, animal scales, syringes, oral sondes, animal restrainers, rat cages, glucometers, glucose test strips, and other glassware. The ingredients used consist of nicotinamide, streptozotocin, Na-CMC, glibenclamide tablets, 96% ethanol, distilled water, aquabides, potassium iodide, iodine, bismuth (III) nitrate, mercury (II) chloride, iron (III) chloride, alpha naphthol, concentrated nitric acid, lead (II) acetate, concentrated hydrochloric acid, sodium hydroxide, concentrated sulfuric acid, acetic acid anhydride, chloralhydrate, amyl alcohol, 2N HCl, isopropanol, chloroform, 0.5 N nitric acid, magnesium powder, toluene, n-hexane, citric acid, sodium citrate.

Sample Preparation

Sampling was carried out purposively, without comparison with the same material from other regions. The material used is Turmeric Leaves (*Curcuma domestica* Val.) Obtained from Tigaras, Kec. Dolok Pardamean, Kab. Simalungun, North Sumatra Province. Plant identification was carried out at the USU FMIPA Pharmaceutical Biology Laboratory. Turmeric Leaves (*Curcuma domestica* Val.) that have been collected are cleaned of impurities, then washed under running water several times until clean, then drained and then spread on parchment until evenly distributed until the water is absorbed; after that, it is weighed as a wet weight then dried in a drying cupboard until dry (marked when it is crushed brittle)—then weighed as dry weight. The dried simplicia was blended into a powder and then stored in a tightly closed container at room temperature.

Examination of Simplicia Characteristics

Characteristics of simplicia include macroscopic and organoleptic examination, microscopy, determination of water content, determination of water-soluble extract, determination of soluble extract in ethanol, determination of total ash content, and determination of acid-insoluble ash content.

Phytochemical Screening

Phytochemical screening includes an examination of alkaloids, flavonoids, saponins, tannins, glycosides, and steroids/triterpenoids.

Preparation of Turmeric Leaf Ethanol Extract (EEDK)

Simplicia powder was extracted by maceration method using ethanol solvent. According to the Indonesian Pharmacopoeia Edition III (1979) [12], the method is as follows: As much as 500 g (10 parts) of simplicia powder is put into a vessel, poured with 3750 mL (75 parts) of ethanol, closed, left for five days protected from light while frequently stirred, sprinkled, squeezed. The dregs are macerated with enough ethanol to obtain 5 L (100 parts). Transfer to a covered vessel, and leave in a cool place protected from light for two days. Elap, pour or strain. Extract concentration was carried out using a rotary evaporator at 40°C, then evaporated in a water bath at 40°C until a thick extract was obtained.

Preparation of Turmeric Leaves Ethanol Extract Suspension (EEDK)

Weigh each EEDK dose of 100, 200, 400 mg/kg bw with a watch glass, then put into a mortar and add 0.5% Na-CMC suspension little by little while grinding until homogeneous, then put into a 10 mL volumetric flask. The volume comprised 0.5% Na-CMC suspension up to the marked line.

Preparation of Test Animals

The animals used were white male rats with a body weight of 180-200 g divided into six groups, each consisting of 4 rats. Prior to the experiment, they were acclimatized for two weeks. From this study,

researchers will use a sample of 24 rats and have received approval from the ethics commission with number 0570/KEPH-FMIPA/2022.

Testing the Antidiabetic Effects of Ethanol Extract of Turmeric Leaves (EEDK)

Rat blood was taken from the tip of the tail; the tail was cleaned with 70% alcohol, then slashed with a razor blade and the blood that came out was attached to a glucometer paper strip that had been attached to the device than the number printed on the screen of the tool was recorded, the rat tail tip scar was treated with 70% alcohol. Twenty-four male rats weighing 180-200 g which had been fasted for 18 hours, were weighed, determined fasting Blood Glucose Level, induced with nicotinamide 230 mg/kg bw intraperitoneally, after 15 minutes, then induced with 65 mg/kg streptozotocin solution intraperitoneally [13]. Rats measured their blood glucose levels on day 5 [14]. Mice are considered diabetic if their fasting blood glucose level is ≥ 200 mg/dL and can be used for testing.

Test the antidiabetic activity using the EEDK, which is given orally once every day. The test animals used in this experiment were white Wistar rats induced by nicotinamide and streptozotocin and divided into six groups. Each group consisted of 4 rats, namely:

1. Group 1: Na-CMC suspension
2. Group 2: EEDK dose of 100 mg/kg body weight.
3. Group 3: EEDK dose of 200 mg/kg body weight.
4. Group 4: EEDK dose of 400 mg/kg body weight
5. Group 5: Glibenclamide dose of 0.45 mg/kg body weight as a positive control
6. Group 6: normal (no treatment)

Each group was given the test preparation orally, and then blood glucose levels were measured on days 4, 8, 12, 16, 20, 24, and 28 [15].

III. RESULT AND DISCUSSION

Results of Examination of Plant Characteristics

The macroscopic examination of fresh turmeric leaves showed that the leaves were 10-40 cm long and 8-13 cm wide. Turmeric leaves are lanceolate (eggs), pinnate, and pale green with a tapered tip, base, and flat leaf edges. The simplicia powder of turmeric leaves has a bitter taste and a distinctive turmeric smell. A microscopic examination of simplicia powder characteristics was conducted to obtain simplicia identity. The microscopic examination of the simplicia powder characteristics showed parenchyma cells filled with essential oils, covering hairs, spiral-shaped wooden vessels, parasitic-shaped stomata and cells filled with oil droplets. The macroscopic and microscopic examination results can be seen in Figure 1.



Fig 1. Macroscopic (a) and microscopic (b) turmeric leaves, 1. Parenchyma cells contain essential oil, 2. Closing hair (Trichoma), 3. Spiral shape of wood vessels, 4. Stomata are parasitic forms and cells contain oil droplets

Results of Simplicia Characterization of Turmeric Leaves

Determination of the water content in the simplicia is carried out to determine the amount of water contained in the simplicia used. The results of determining the water content obtained are less than 10%, namely 2.65%. Moisture content that exceeds 10% can be a suitable medium for microbial growth and the presence of fungi and encourages damage to the quality of simplicia [16]. Determination of extract content

was carried out using two solvents, namely water and ethanol. The determination of the water-soluble and ethanol-soluble extracts was aimed at determining the levels of the active compounds extracted in the solvent from several simplicia powders. The results of determining the water-soluble essence content of turmeric leaves were 30.64%, while the ethanol-soluble extract content was 69.83%. Determination of total and acid-insoluble ash content aims to assure that the simplicia does not contain certain heavy metals exceeding the values set for plant simplicia because they can be harmful (toxic) to health. Determination of the total ash content states the amount of inorganic compound content in the simplicia, for example, Mg, Ca, Na, Zn and K. The ash content is insoluble in acid to determine the levels of inorganic compounds that are insoluble in acid, for example, silicates. Total ash is divided into two, namely physiological ash and non-physiological ash. Physiological ash is ash that comes from the plant tissue itself. In contrast, non-physiological ash is the residue after burning from external materials found on the surface of the simplicia [16]. Determination of the ash content of turmeric leaf simplicia showed a total ash content of 7.21% and an acid-insoluble ash content of 1.60%. Determination of the total ash content of turmeric leaf simplicia was obtained at 7.21%; the high ash content in simplicia was because turmeric leaves contain the minerals potassium, sodium, calcium, and magnesium. The calculation results of simplicia characterization can be seen in Table 1.

Table 1. Results of simplicia characterization of turmeric leaves

Parameter	Result
Water content	2.65%
Water soluble essence content	30.64%
Ethanol soluble essence content	69.83%
Total ash content	7.21%
Acid insoluble ash content	1.60%

Results of Phytochemical Screening

The results of the phytochemical screening of turmeric leaf simplicia powder (*Curcuma domestica* Val.) were carried out to obtain information on the class of secondary metabolites. The results of the phytochemical screening can be seen in Table 2.

Table 2. Results of phytochemical screening of turmeric leaf simplicia

Secondary Metabolites	Reactants	Result
Alkaloids	Dragendorff	-
	Bouchardat	-
	Meyer	-
Flavonoids	Mg Powder + Amyl Alcohol + HCl _p	+
Glycosides	Molish+H ₂ SO ₄	+
Saponins	Hot/shaken water	-
Tannins	FeCl ₃	+
Triterpenes/Steroids	Lieberman-Bourchat	+

Information: (+) positive = contains a group of compounds, (-) negative = does not contain a group of compounds

Phytochemical examination showed the presence of flavonoid compounds with the addition of magnesium powder and concentrated hydrochloric acid to produce a red solution—examination of glycoside compounds with Molisch and concentrated sulfuric acid to form a purple ring. The addition of FeCl₃ gives a blackish-green colour, indicating the presence of a class of tannin compounds [17]. Examination of the triterpenoid/steroid compound groups by adding a few drops of Liebermann-Burchard reagent produces a pink or purple colour which indicates the triterpenoid compound group [18]. Examination of the class of alkaloid compounds was carried out by adding Mayer, Bouchardat and Dragendorff reagent solutions which did not show any lumpy white precipitate, blackish-brown precipitate, or red solution. This shows that turmeric leaves do not contain alkaloid compounds. Samples with hot distilled water and shaking vigorously and then adding 2N HCl did not produce stable foam, indicating the absence of saponin compounds [17].

EEDK Antidiabetic Activity Test Results

The average Blood Glucose Level was measured in mice fasted for 18 hours; then, the measured Blood Glucose Level was called the normal Blood Glucose Level average or the average Blood Glucose Level before being induced by NA and STZ. The normality test results obtained a significant value of 0.260

at $\alpha = 0.05$, indicating no significant difference between the control group, the test group and the comparison group. This shows that the experimental animals were used in homogeneous physiological conditions, namely in average blood glucose levels, to be used as test animals. The results of the rats' Blood Glucose Level measurements before and after induction can be seen in Figure 2.

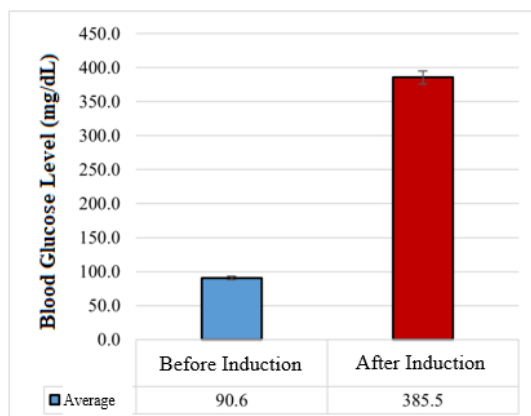


Fig 2. Blood glucose level of rats before and after NA and STZ induction
 Description: Blood glucose levels before and after NA and STZ induction.
 (Data presented as mean, SEM, analyzed by paired sample t-test, n=20).

Based on Figure 2, it can be seen that the administration of 230 mg/kg bw of NA and 65 mg/kg bw of STZ for all experimental animals resulted in Blood Glucose Level ≥ 200 mg/dL. This shows that the rats used for the experiment were in a state of hyperglycemia. The results of the statistical test using the paired sample t-test showed a significant difference in Blood Glucose Level before and after induction, namely 0.000 at $\alpha = 0.05$. This shows that the induction of NA and STZ affected increasing the blood glucose levels of the test animals. Mice with Blood Glucose Level ≥ 200 mg/dL are referred to as diabetic rats.

The treatment was started after the rats tested positive for diabetes (day 0), then every day, they were given the test preparations in each test group for 27 days, and the Blood Glucose Level was measured on the 4th, 8th, 12th, 16th, 20th, 24th and 28. The Blood Glucose Level data (mg/dL) of each group of rats, the average of the Blood Glucose Level between individuals was calculated, then statistically analyzed using ANOVA and continued with the Post Hoc Tukey HSD test to see significant differences between treatments. The greatest percentage of reduction in Blood Glucose Level from the start of administration of the extract to the 28th day occurred in the positive control group (glibenclamide). Namely, by 60.54%, EEDK 400 mg/kg bw decreased by 58.13%, EEDK 200 mg/kg bw decreased by 49.34%. EEDK 100 mg/kg body weight decreased by 37.96%. Whereas in the negative control group (Na-CMC), there was an increase in Blood Glucose Level. This can be seen from the bar graph that points downward (to the minus number), which is -8.24%. The graph of the percentage decrease in Blood Glucose Level can be seen in Figure 3.

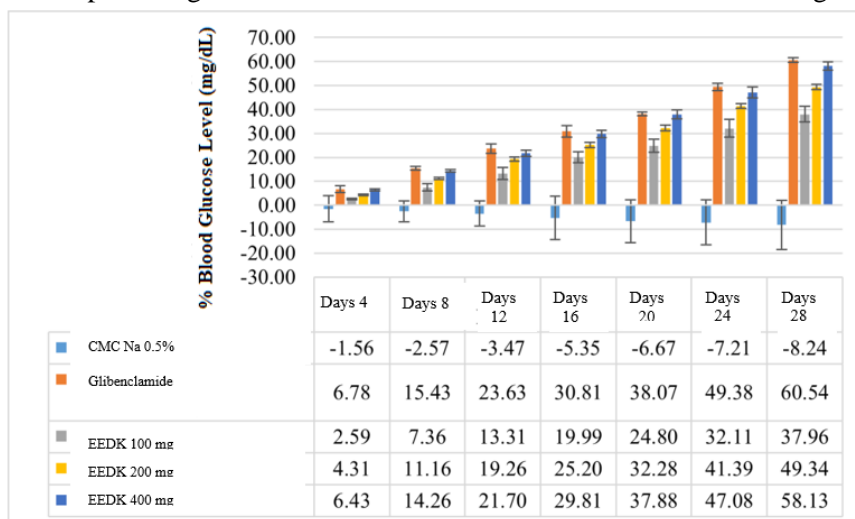


Fig 3. Graph of decreased blood glucose levels

Increasing the dose of ethanol extract from turmeric leaves showed increased hypoglycemic activity. The higher the turmeric leaf ethanol extract dose used, the greater the effect of reducing blood glucose levels produced. This indicates that the components of the active chemical compounds contained in the ethanol extract of turmeric leaves have a synergistic effect. Velazquez et al. (2011) stated that complementary alternative medicines derived from nature synergise in treating a disease [19]. Based on the results obtained, administration of 100 mg/kg bw EEDK suspension, 200 mg/kg bw EEDK, 400 mg/kg bw EDK, and 0.45 mg/kg bb glibenclamide proved to have a reduced effect on Blood Glucose Level until the 28th day, except for the negative control. This is due to the induction of STZ; STZ is a diabetogenic chemical compound that damages pancreatic β cells directly. STZ cytotoxicity causes the release of free radicals, which trigger intracellular oxidative stress.

STZ penetrates Langerhans β cells via glucose transporters GLUT 2. The intracellular action of STZ results in changes to pancreatic β -cell DNA [20]. The content of secondary metabolites in turmeric leaves, such as flavonoids, tannins, glycosides, and steroids, is essential in lowering blood glucose levels. Flavonoids can reduce blood glucose levels by inhibiting gluconeogenesis by increasing the ratio of AMP, thereby activating AMPK, increasing glucose uptake in skeletal muscle and adipose tissue, increasing insulin sensitivity, increasing insulin secretion, reducing apoptosis, increasing proliferation pancreatic β cells, inhibit the α -glucosidase enzyme, inhibit glucose absorption and increase glucose tolerance [6, 9, 21]. The mechanism of tannins lowers blood glucose levels, namely increasing glucose absorption through activation of insulin signalling pathway mediators, GLUT-4 translocation, regenerating pancreatic β cells, increasing insulin activity, increasing glycogenesis, as an astringent substance that can cause wrinkling of epithelial membranes intestine thereby reducing the absorption of food [22, 23].

IV. CONCLUSION

Based on the discussion and research results, it can be concluded that turmeric leaves contain secondary metabolites, namely flavonoids, glycosides, tannins, and triterpenoids/steroids, but there are no alkaloids and saponins. The characterization results show that the sample meets the requirements. Turmeric leaf ethanol extract doses of 400 mg/kg, 200 mg/kg, and 100 mg/kg could reduce the Blood Glucose Level of NA and STZ-induced rats from the fourth day of treatment.

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