

ANTIOXIDANT POTENTIAL OF GOTU KOLA LEAF EXTRACT (*Centella asiatica* (L.) Urban) AS AN ALTERNATIVE TO ANTIHYPERGLYCEMIC HERBAL DRINKS

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Received: October 4, 2022. Accepted: November 4, 2022. Published: November 30, 2022

Abstract: This study aims to determine the antihyperglycemic potential of Gotu kola leaf extract (*Centella asiatica* (L.) Urban) as an alternative herbal drink in diabetic male white mice. This study was divided into four stages: extracting Gotu kola leaves using 96% ethanol as solvent, phytochemical screening, and testing antihyperglycemic activity. Male white mice were made diabetic by alloxan-induced. Mice were divided into five groups consisting of negative control (Aquadest), positive control (Glibenclamide 13 mg/Kg BW), dose 1 (200 mg Gotu kola extract/kg BW), dose 2 (400 mg Gotu kola extract/kg BW), dose 3 (600 mg Gotu kola extract/kg BW). The results of phytochemical screening showed that the Gotu kola leaf extract was positive for flavonoids and tannins. The analysis results on the Gotu kola leaf extract showed an IC50 value of 80.58 ppm, including strong antioxidant activity. The results of the antihyperglycemic activity test showed that diabetic male white mice were found to have decreased blood sugar levels on days 14 and 21 by respectively 24.3% and 41.6%; 25.4% and 42.3%; 37.8% and 55.3% after administration of Gotu kola leaf extract at P1, P2, and P3.

Keywords: *Centella asiatica* (L.) urban, Antioxidant, Antihyperglycemic

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by an increase in blood glucose levels exceeding normal (hyperglycemia) and disturbances in carbohydrate, fat, and protein metabolism associated with a lack of sensitivity or insulin secretion and progressive changes in the structure of pancreatic beta cells. Hyperglycemia in uncontrolled DM patients can cause severe damage to body tissues, such as nerves and blood vessels. People with DM require lifelong treatment to reduce symptoms, prevent disease progression and prevent complications from developing. Antioxidants can inhibit oxidation reactions by binding to free radicals and highly reactive molecules. Free radicals can damage cell-forming macromolecules that can cause degenerative diseases. Humans have endogenous antioxidants in the body that can reduce free radicals. However, if the amount is smaller than free radicals, exogenous antioxidants are needed to minimize the harmful effects of free radicals [1].

One factor that influences the decrease in blood glucose levels is adherence to diabetes mellitus treatment therapy. Therefore adherence is closely related to blood glucose levels. The higher the level of patient compliance, the blood sugar level will decrease. On the contrary, the lower the patient's medication adherence, the blood glucose level cannot be controlled, which means that the blood glucose level will remain high [2]. Public knowledge of medicinal plants is very diverse, from processing, how to use, the parts used, and the efficacy of each type of plant in curing disease. Of

the several medicinal plants obtained in the field, not all can cure various diseases [3]. Gotu kola is one of the plants that are easy to grow and is often found in plantations, roadside fields, ripening rice fields, or wet fields. People also know this plant as one of the plants for fresh vegetables. Based on the results of interviews with the community, Gotu kola is also often used by the community as cough medicine and minor healing wounds. Community knowledge about other benefits of Gotu kola is very minimal [4]. People affected by diabetes mellitus often consume synthetic drugs and are very dependent. Synthetic drugs consumed by the public tend to have fewer therapeutic effects than side effects, and most synthetic drugs cause side effects that are unacceptable to the body [5].

Natural antioxidants such as ascorbic acid, carotenoids, tocopherols, phenolic compounds, and flavonoids are safe alternatives to synthetic antioxidants. Flavonoid compounds can counteract free radicals and have biological activities such as inhibiting cancer cells, anti-inflammatory, anti-viral, anti-fungal, and antibacterial [6]. Gotu kola is a wild plant that Indonesian people have long consumed as a traditional medicinal herb. Gotu kola contains active compounds such as saponins, asiatic acid, acicosides, madecacosides, triterpene acids, carotenoids, salts of K, Na, Ca, Fe, phosphorus, vallerine, tannins, resins, pectin, sugars, and B vitamins [7]. Gotu kola has benefits as an antioxidant as well as antibacterial, increasing memory activity, overcoming inflammation, providing a calming effect, and improving mental function for the better [8].

The Gotu kola plant is widely used as a medicinal plant, fresh vegetables, fresh vegetables, or juice. Scientific research has shown the efficacy of Gotu kola, including antineoplastic effects, protective effects of gastric ulcers, lowering vessel wall pressure, accelerating wound healing, increasing appetite, fever, snakebite, refreshing the body, reducing fever, dry cough, nosebleeds, increasing intelligence, and antithrombosis. In addition, Gotu kola leaves can also be used as a medicine for diabetes [9]. The ethanol extract of Gotu kola leaf was reported as antidiabetic because it suppresses the increase in blood sugar levels in alloxan-induced rats [10]. The Gotu kola plant contains antioxidants in all parts of the plant, from leaves to roots [11]. The triterpene aglycone compound in Gotu kola is non-polar, which when it binds to 3 sugar molecules, it still has little solubility in water and is more soluble in 96% ethanol. The extraction process using ethanol is proven to be better than methanol and water. Ethanol can dissolve triterpene aglycone compounds well, especially the phenolic content, asiaticoside, and madecassoside [12].

DM management is effective in the early stages before symptoms or prediabetes occur. One way to control blood glucose levels can be done in a traditional way using natural ingredients. The Gotu kola leaf contains many active substances, including tannins and flavonoids. Tannins function as antihyperglycemic agents by preventing glucose uptake at the brush border in the small intestine. At the same time, flavonoids are alpha-glucosidase inhibitors that delay carbohydrate absorption so that blood glucose will decrease. In addition, wuluh starfruit contains several vitamins and minerals including riboflavin, vitamin B1, niacin, ascorbic acid, carotene, vitamin A, while the minerals include phosphorus, calcium, and iron.

This study aims to analyze the antioxidant potential of Gotu kola leaf extract (*Centella asiatica* (L.) urban) as an alternative to antihyperglycemic herbal drinks. This research is also useful in providing information to the public about the efficacy of wild plants in lowering blood glucose levels.

RESEARCH METHOD

Gotu kola Leaf Extract

The dried simplicia of Gotu kola (*Centella asiatica* (L.) Urban) was cleaned and then crushed. The powder is then put in a container, macerated with ethanol solvent, and allowed to stand for a certain time. The macerate is separated by filtration and repeated at least twice with the same amount and type of solvent until the solvent is clear. All the macerates were collected, and the solvent was evaporated using a rotary evaporator to obtain a thick extract. Gotu kola leaves that have been dried and reduced in particle size are then extracted using

96% ethanol [13]. The extractant was filtered and thickened using rotary vapor to produce Gotu kola leaf extract [14].

Phytochemical Screening

Gotu kola leaves were then screened for their phytochemical content in alkaloids, flavonoids, tannins, and saponins and analyzed for antioxidant activity.

Antioxidant Activity (DPPH Method)

Preparation of DPPH solution by weighing 5 mg of DPPH and then adding methanol p.a up to 50 mL. Determination of the maximum absorption wavelength of DPPH by determining the optimum wavelength, measuring the absorbance at a wavelength of 510-525 nm. The mother liquor was made in series with concentrations of 4, 8, 12, 16, 20, and 100 ppm. Determination of % Inhibition and IC₅₀.

$$\% \text{ Inhibition} = \frac{\text{Abs. blank} - \text{Abs. sample} \times 100\%}{\text{Abs. Blank}}$$

Treatment of Experimental Animals

Male white mice that had been induced by alloxan were divided into five groups and given intake of aquadest (control), glibenclamine 13 mg/Kg BW (positive control), Gotu kola extract 200 mg/Kg BW (P1), 400 mg/Kg BW (P2) and 600 mg/Kg BW (P3). Mice were measured for blood sugar content on days 7:7-14 and 21:7-21 [15].

RESULT AND DISCUSSION

Phytochemical Screening and Antioxidant Activity

The results of the phytochemical screening of Gotu kola leaf extract showed that the samples tested positive for flavonoids and tannins. The results of the phytochemical screening analysis and antioxidant activity are shown in Table 1.

Table 1. Phytochemical Screening Analysis

No.	Identification of compounds	Result
1.	Flavonoid	+++
2.	Tanin	+
3.	Alkaloid	++

The results of this study indicate that Gotu kola leaf extract contains alkaloids, flavonoids, tannins, and saponins. Gotu kola contains various active ingredients: triterpenoid saponins, triterpenoids genin, essential oils, flavonoids, phytosterols, flavonoids, vitamin A and vitamin C, and tannins [16]. Flavonoids are one of the largest groups of natural phenolic compounds in plants and are composed of 15 carbon atoms as the basic nucleus. Composed of the C6-C3-C6 configuration, there are 2 aromatic rings connected by three

carbon atoms which may or may not form a third ring. Gotu kola contains active flavonoid compounds that protect leaf tissue damage and cells from ultraviolet radiation [17]. Gotu kola leaves, besides containing flavonoids, also contain alkaloids. Alkaloids are the most common secondary metabolites with nitrogen atoms in plant tissues [18]. Alkaloids in Gotu kola leaves function as medicine, neutralize toxic substances, detoxify metabolic products, regulate growth and provide nitrogen elements needed by plants, anti-diarrhea, anti-diabetic, anti-microbial and anti-malarial.

Tannins are polyphenolic compounds with a fairly high molecular weight (more than 1000) and

can form complexes with proteins. Tannins have several health benefits, namely as astringent, antidiarrheal, antibacterial, and antioxidant [19]. The antioxidant activity test was carried out by making a DPPH solution which was then tested to determine the maximum wavelength—the results of the determination of the length. The maximum waveform of DPPH obtained is 515 nm. After obtaining the wavelength maximum, the next step is to measure the absorbance of the sample for each concentration and calculate the % inhibition. The results of the absorbance and % inhibition measurements obtained can be shown in Table 2.

Table 2. Absorbance and % Inhibition of Gotu kola Leaf Extract

Material	Concentration (ppm)	Abs. Blank	Abs. Sample	% Inhibition
Gotu kola leaf extract	4	0.71	0.631	12.86
	8		0.642	11.37
	12		0.621	12.89
	16		0.619	13.26
	20		0.613	14.33
	100		0.261	62.76

Table 3. IC₅₀

Material	IC ₅₀ (ppm)
Gotu kola leaf extract	80.58

Table 2 shows that the greater the concentration of the sample solution, the smaller the absorbance value obtained. The smaller the absorbance value, the greater the % inhibition value. It is because the higher the concentration of the solution, the higher the antioxidant activity.

The amount of antioxidant activity is indicated by the IC₅₀ value, which is the concentration of sample solution required to inhibit 50% of DPPH free radicals. Table 3 shows the IC₅₀ value in Gotu kola leaf extract of 80,58 ppm, which is included in the strong antioxidant activity.

Antihyperglycemic Activity

The test results showed that the extract had antihyperglycemic activity. Male diabetic white mice experienced a decrease in blood sugar content on the 14th and 21st days after administration of Gotu kola leaf extract in treatments P1, P2, and P3 by 24.3% and 41.6%, respectively; 25.4% and 42.3%; 37.8% and 55.3%. The results of the decreased measurement are shown in Table 4.

The group of mice given Gotu kola leaf extract experienced a decrease in blood sugar levels. The decrease in blood sugar levels in mice is thought to be due to the presence of phytochemical compounds in the Gotu kola leaf extract. Flavonoids, alkaloids, tannins, and saponins have shown their antidiabetic properties.

Table 4. Lowering Blood Sugar Levels

Group	Day-14 (%)	Day-21 (%)
Positive Control	38.6	54.2
P1	24.3	41.6
P2	25.4	42.3
P3	37.8	55.3

The antidiabetic action mechanism of phytochemicals found in plants can be categorized into six groups based on their pharmacological mechanisms: glucose metabolism modulator, hypolipidemic effector, pancreatic effector, antioxidant effector, the modulator of diabetes-related complications and insulin-mimetic modulator and insulin-sensitizer. Phytochemical compounds in the form of flavonoids, alkaloids, tannins, and saponins can modulate metabolic pathways where glucose can act as a substrate or product. These phytochemical compounds affect gluconeogenesis, glycogenolysis, pentose phosphate pathways, and glycogenesis. These phytochemical compounds also work by interfering with glucose absorption and inhibiting the activity of -glucosidase and -amylase. The next mechanism is that phytochemical compounds are thought to be able to reduce triglyceride levels and cholesterol

content, which will have an impact on hyperlipidemia, which is one of the pathophysiological features of DM [20].

Another mechanism is that bioactive compounds such as flavonoids and alkaloids can protect against cell damage, increase proliferation and stimulate insulin secretion. Flavonoids, alkaloids, tannins, and saponins can protect themselves from oxidative stress associated with diabetes complications. Meanwhile, alkaloids can stimulate cellular glucose absorption and reduce insulin resistance. Tannins also have an antidiabetic effect with the mechanism of action of increasing the propagation of cell recovery and reducing carbohydrate absorption by inhibiting the activity of α -amylase and α -glucosidase. Meanwhile, saponins display their antidiabetic activity through possible mechanisms of improving insulin resistance, stimulating insulin secretion, and protecting pancreatic cells. The antioxidant activity of Gotu kola will complement the body's defense system to ward off free radicals to limit the damage that occurs. The antioxidant defense system works by interacting directly with free radicals to prevent the formation of reactive oxygen compounds or changing reactive compounds to be less reactive [21-24].

CONCLUSION

The antioxidant activity of Gotu kola leaf extract (*Centella asiatica* (L.) Urban) leaf extract is 80,58 ppm which is included in the strong antioxidant activity. Gotu kola leaf extract contains antioxidants that can be used as an alternative to antihyperglycemic herbal drinks.

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