International Journal of Public Health
Volume. 1 Number. 4 Year 2024
e-ISSN: 3047-5228; dan p-ISSN: 3047-5236, Hal. 211-220
DOI: https://doi.org/10.62951/ijph.v1i4.275
Available online at : https://international.arikesi.or.id/index.php/IJoPH

Antidiabetic Activity Test of Ethyl Acetate Fraction of Takokak Fruit *(Solanum torvum Swartz)* Using Oral Glucose Tolerance Test (OGTT) Method

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Abstract *Takokak fruit is one of the plants that can be used to lower blood sugar levels. This study aims to identify the bioactive compounds of ethanol extract of takokak fruit and test the antidiabetic ethyl acetate fraction. Antidiabetic testing was carried out by in vivo testing using the Oral Glucose Tolerance Test (OGTT) method to see the decrease in blood glucose levels using white rats as test animals. The test results showed that takokak fruit contains flavonoids, steroids, and tannins. Fractionation is the process of extracting compounds from the extract using two types of solvents that do not mix with each other. The results of the antidiabetic test showed that the ethyl acetate fraction with glibenclamide as a comparator had the ability as an antidiabetic measured by the amount of difference in the decrease in blood glucose levels in the test animal group T_{30} to T_{120}. From the results obtained, the average decrease in blood glucose levels in the negative control group (Na CMC) was 5.8 mg/dL, the positive control group (Glibenclamide) 21.03 mg/dL, and the fraction group 9.86 mg/dL. Takokak fruit has antidiabetic activity. Compounds that have antidiabetic activity are flavonoids, tannins, and steroids.*

Keywords: *Takokak Fruit, Antidiabetic, TTGO*

1. INTRODUCTION

Takokak *(Solanum torvum Swartz)* is a plant that grows and is widely distributed in Indonesia. In South Asia and Southeast Asia, the fruit is used as a vegetable and has medicinal properties to improve blood circulation, relieve pain (analgesic), and relieve coughs (antitussive). Utilization of the takokak plant It is common in various regions, one of which is on the island of Java, but not many people know about the efficacy of this plant as a medicine . In general, the takokak plant is also one of the plants included in the genus *Solanum L.* which contains secondary metabolites in the form of alkaloids, flavonoids, saponins, quinones, tannins, triterpenoids . In South Asia and Southeast Asia, raw takokak fruit can be consumed as vegetables or raw vegetables (Martina et al., 2021) .

Takokak fruit is commonly used in traditional medicine (Agrawal et al., 2010) . Takokak fruit also has antidiabetic activity. Antidiabetic is an activity provided by certain compounds that can treat diabetes. Diabetes mellitus is a serious and chronic metabolic disorder with various severe consequences, both acute and chronic. It is estimated that around 25% of the world's population is affected by this disease due to environmental and genetic factors. Diabetes triggers long-term destruction, dysfunction, and failure of various organ systems (heart, blood vessels, eyes, kidneys, and nerves), causing disability and premature death. The severity of damage triggered by

Received: October 30 2024; Revised: November 30,2024; Accepted: December 28,2024; Published: December 30, 2024

hyperglycemia in each organ system may be related to how long the disease has been present and how well it has been controlled (Salehi et al., 2019) .

Population growth, inadequate drug supply, high cost of treatment, side effects of some drugs, and development of resistance to currently used drugs.

has led to an increased emphasis on the use of plant materials as a source of medicine for various diseases, one of which is using the takokak plant as a source of traditional medicine (Manikandaselvi S, 2018). The method that can be used to test antidiabetic inhibitory activity is the Oral Glucose Tolerance Test (OGTT) method.

Oral Glucose Tolerance Test (OGTT) is a test used to confirm the diagnosis of diabetes mellitus (Wati, 2020). OGTT is carried out by inducing glucose orally so that it can stimulate insulin secretion in order to regulate blood glucose levels within the normal range (Juananda et al., 2021). Basically, the OGTT method is a test to see the decrease in blood sugar levels in test animals (Sangkal, 2021). In general, this is done using samples from natural materials, namely takokak fruit which is extracted after which fractionation is carried out.

Fractionation is a method of separating extracted compounds using two types of solvents with different polarities. Common solvents used for fractionation are n-Butanol to attract non-polar compounds, ethyl acetate to attract semi-polar compounds, while methanol to attract polar compounds (Irwan, 2017).

2. RESEARCH METHODOLOGY

Making Thick Extract

Simplisia was weighed as much as 300 grams, put into a container (glass jar) extracted through the maceration method (1:7.5 g/mL) using 95% ethanol for seven days. The use of a rotary evaporator to separate the resulting macerate from the solvent. Ethanol-free test in takokak fruit extract, namely the extract is added with CH_3COOH, then heated. The absence of a distinctive ether odor indicates a negative test result. The second part is dissolved in 200 mL of distilled water (1:9) then partitioned using ethyl acetate solvent. To obtain a thick fraction of the filtrate from the fractionation results, it is evaporated which will then be used for antidiabetic testing (Sangkal, 2021) .

Fractionation Making

Ethanol extract 95% was dissolved in distilled water and stirred until dissolved, the extract was mixed then water was put into a separating funnel and fractionation was carried out using ethyl acetate solvent with a ratio of 1: 9, the results of the fraction were stored and the residue obtained was added with distilled water, then the solvent was evaporated from the fraction results with *a rotary evaporator*. Furthermore, the ethyl acetate fraction that had been obtained was weighed and its immersion value was calculated (Hasanah, 2019).

Making Glucose Solution

Glucose was weighed as much as 50g and put into a 100 ml *beaker glass* , then suspended with 100 ml of 1% Na-CMC until homogeneous (Khairiani, 2018).

Preparation of Test Animals

Experimental animals: white mice weighing around 150-200 grams, aged 3-4 months, totaling 9 were kept in a cage. The mice were adapted for 2 weeks to reduce the impact of stress that would affect the body's metabolism because they were in a new environment and could disrupt the study. Throughout the process of adaptation, the mice were always given food and drink *ad libitum* .

Grouping of Test Animals

This study used 9 white mice weighing 102-170 grams which were divided into 3 groups. Before the experiment was carried out, the test animals were first adapted to provide comfort to the new environment, the test animals were fasted for approximately 12 hours and were still given water before the treatment was carried out (Sangkal, 2021).

Antidiabetic Activity with TTGO Method

Before being given treatment to each group of experimental animals, they were first fasted for 12 hours (still given water). The weight of the experimental animals was weighed and marked, then fasting blood sugar was checked using *a glucose test glucometer , test strips* via the rat's tail vein. Glucose was induced orally in each rat. After 30 minutes, blood sugar levels were measured after induction. The next stage was to measure glucose levels at minute 60 (T_{60}), minute 90 (T_{90}), minute 120 (T_{120}) (Sangkal, 2021).

Treatment of Test Animals

The first treatment, all experimental animals will be fasted for 12 hours. After that the initial/fasting blood glucose levels are measured. Furthermore, test samples are given, namely

Group 1 induced by 1% Na-CMC as a *negative control* , group 2 given glibenclamide as a comparison (*positive control*), group 3 given 10% ethyl acetate fraction orally after which the blood glucose levels of white mice are measured every 45 minutes for 3 hours of observation. White mice are considered to have diabetes when blood glucose levels are> 200 mg / dL (Nurhidajah et al., 2017) .

Calculation of the dose to be dispersed:

Takokak fruit fraction 10% = $\frac{10}{100}$x 20 = 2 ml

Example: Rat BB 162 —162 / 200 x 2g = 1.62 g

Given volume = 162 g / 2 g x 2 ml = 1.62 ml

Making takokak fruit extract solution

Weigh 2 grams of takokak fruit extract, dissolve in 20 ml of Na-CMC suspension, to obtain a 10% extract.

Preparation of stock solution

Dissolve 1000 mg/1 g of Na-CMC in 50 ml of distilled water (70°C) and then leave it for half an hour until a transparent mass is obtained, after which 100 ml of distilled water is added to dilute it (Wardani, 2016).

Secondary Metabolite Test

Ethanol extract of takokak fruit was used as a sample for phytochemical screening, with each test.

Alkaloid Test

Evaporated 2 mL of extract in a porcelain cup. The precipitate was then dissolved with 5 mL of HCl. The solution obtained was divided into 3 test tubes. The first tube as a blank, by adding 3 drops of HCl, 3 drops of dragendorff reagent were added to the second tube and 3 drops of mayer reagent to the third tube. The positive test result for alkaloids is if there is a color change in the reagent, namely dragendorff forms an orange precipitate and mayer reagent forms a yellow precipitate.

Flavonoid Test

Magnesium powder as much as 0.05 mg and 1 mL of concentrated HCl were added to 2 mL of extract, let stand for 5 minutes then filtered and shaken vigorously. The test is positive if it shows red, yellow, or orange color.

Tannin Test

A few drops of $FeCl_3$ are added to 1 mL of the extract. A positive tannin test is indicated by the formation of a dark blue or blackish green color.

Steroid Test

10 drops of CH_3COOH were added to 1 mL, shaken and left for a few minutes. Positive steroids if there is a blue or green color change.

Terpenoid Test

As much as 2 drops of H_2SO_4 are put into 1 mL. Leave it for a few minutes then shake it, the test result is positive if a red or purple color change forms.

Saponin Test

2-3 mL of extract is put into a test tube, and 10 mL of hot water is added and then cooled, shaken vigorously for 10 seconds then 1 drop of HCl is added. Positive results if stable foam is formed as high as 1-10 cm for no less than 1 minute (Grace et al., 2014).

Data analysis

The data analysis used in this study is descriptive analysis, the data obtained will be displayed in tables and graphs.

3. RESULTS AND DISCUSSION

The extraction results of 300 grams of takokak fruit *(Solanum torvum Swartz)* with 95% ethanol obtained a thick extract of 19.6 grams and was divided into two parts for phytochemical screening and fractionation testing followed by TTGO (Oral Glucose Tolerance Test). Extraction results
can be seen in table 1

Table 1 Results of Takokak Fruit Extract

Sample (g)	Sample Weight (g)	Extraction Results (g)	Color	Soaking Results %
Takokak fruit (*Solanum torvum Swartz*)	300	19.6	Blackish green	6.5

Based on table 1, the thick extract obtained was 19.6 grams with a yield of 6.5%. A good yield according to the Indonesian Herbal Pharmacopoeia is <7.2, while the yield produced was 6.5%. Based on research by Wijaya et al., (2022) stated that if the extraction is carried out for a

long time, the yield will be high. This is because the opportunity to react between the material and the solvent will be long, so that more compounds diffuse out of the cell.

Secondary Metabolite Screening Testing

The screening results of the compounds of ethanol extract of takokak fruit include tannins, steroids, and flavonoids. Color changes occur due to organoleptic chemical reactions that cause the formation of sediment in the extract that has been extracted with the reagent. The screening results of takokak fruit extract can be seen in table 2

Table 2 Results of identification of secondary metabolite compounds of takokak fruit extract

Compound	Reagent	Observation	Results Observation	Literature
Alkaloid	Ammonia + Chloroform + H_2SO_4 + Mayer's reagent	No sediment	(-)	White sediment (Mondong *et et al.,* 2015)
	ammonia + Chloroform + H_2SO_4 + Reagent Wagner	No sediment	(-)	Red sediment Orange (Mondong *et al.,* 2015)
	Ammonia + Chloroform + H_2SO_4 + Reagent Dragendorph	No sediment	(-)	Brown sediment (Mondong *et al.,* 2015).
Flavonoid	Powder Magnesium + Concentrated HCL	Happen color formation Orange	(+)	Produce yellow, orange to Red (Mondong *et al.,* 2015).
Saponins	Aquadest	Stable foaming	(-)	Stable foaming no less from 10 minutes (Mutmainnah,

				2017).
Tannin	Fe CL $_{3\ 10}$ %	The occurrence color formation blackish blue	(+)	Produce green or blue Black (Ergina, 2014)
Steroid	HCL Concentrate + Lieberman Buchard	The occurrence color formation Green	(+)	Produce green (Ergina, 2014)
Terpenoid	CH3COOH + Lieberman Buchard	The formation of the ring Brownish	(-)	The formation of Ring Brownish (Latifah, 2015).

Information :

a. (+) Contains chemical compounds
b. (-) Does not contain chemical compounds

Identification of alkaloid compounds of takokak fruit extract was declared negative because no precipitate was formed in the extract added with Mayer, Wagner, and Dragendorff reagents. Identification of saponin compounds of takokak fruit extract indicated a negative result because no foam or foam appeared for less than 10 minutes. Identification of terpenoid compounds of takokak fruit extract also showed negative results which were indicated by the absence of a brown ring formation.

Antidiabetic Activity Test Results

Antidiabetic activity test was conducted on white rats *(Rattus norvegicus)* using ethyl acetate fraction of takokak fruit with Oral Glucose Tolerance Test (OGTT). From the results of measuring the average blood sugar levels of experimental animals before and after receiving treatment minutes T_0 to T_{120}. All treatment groups had normal blood sugar levels at T_0 based on normal blood sugar levels. is 75-150 mg/dL (Samsuri et al., 2020). The test results can be seen in table 3

Table 3 Blood Glucose Levels of Test Animals

Treatment group	Average blood sugar level mg/dL					
	Fasting blood sugar	After induction	T 30	T 60	T 90	T 120
Na-CMC (-)	95	122	117.3	116.3	111.6	109.3
Glibenclamide (+)	102.3	119	102.3	99.6	92 ,6	82
Takokak fruit fraction 10 %	81	113	107.3	104	100.3	95.6

Based on the table above, there is a blood sugar level in mice after being induced by glucose. Excessive glucose consumption causes *beta cells* to not function optimally in producing insulin hormones, this will then cause a response.

high blood glucose levels (Salma et al., 2013). The negative control blood glucose levels did not experience a high decrease or increase after being given Na-CMC without any treatment, either glibenclamide or extract from time T_0 to T_{120}. At the 30th minute of the first treatment to 120 minutes of the last treatment after being given a negative control sample of 1% Na-CMC, a positive control of glibenclamide, and a 10% ethyl acetate fraction, the results of blood glucose levels were obtained sequentially, namely (K- = 117.3; 116.3; 111.6; 109.3 mg/dL), (K+ = 129.6; 99.6; 92; 82 mg/dL), (K= 158.3; 134.6; 98; 79.6 mg/dL). There was a slight decrease in blood sugar levels in the negative group given 1% Na-CMC which proves that the ability of Na-CMC is a carrier that has no pharmacological effect or has no effect on lowering blood glucose levels, but there is metabolism in the body of mice and diuresis so that blood glucose levels in the body of mice are reduced (Jangga and Suriani, 2016). The percentage of decrease in blood sugar levels can be seen in table 4

Table 4 Blood Sugar Level Reduction Percentage

Treatment Group	Percentage of Blood Sugar Level Decrease (%)				Average (%)
	T 30	T 60	T 90	T 12O	
Na-CMC (-)	3.8	4.6	4.6	10.4	5.8
Glibenclamide	14.03	16.30	22.68	31.09	21.02
FK 10%	5.04	7.9	11.2	15.3	9.86

Based on table 4 above, the percentage of reduction in blood sugar levels for all test groups at 30, 60, 90, and 120 minutes during 2-hour observation increased. The average percentage of decrease in blood sugar levels can be seen in Figure 1

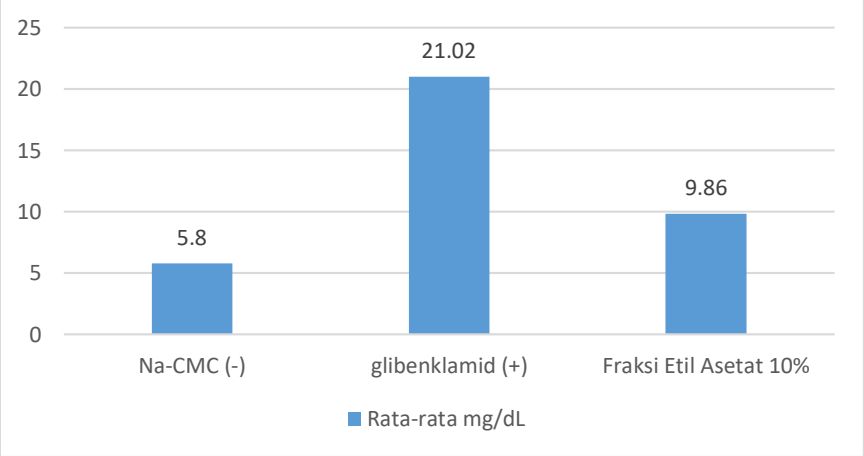

Figure 1 of Average Percentage of Decrease in Blood Glucose Levels of White Mice

Based on the table above, there is a significant difference in the decrease in blood glucose levels in mice between the negative control group and the positive control group and the ethyl acetate fraction group. In the positive control, the decrease in glucose levels was 21.02 mg/dL, 15.6 mg/dL for the negative group, and 9.86 mg/dL for the 10% ethyl acetate fraction. Showing that takokak fruit extract and its fractions along with glibenclamide have the ability to reduce blood sugar levels. The glibenclamide test group had the greatest difference in reduction when compared to the other test groups. The longer the time, the greater the difference in blood glucose reduction, when monitoring is not carried out, there is a greater chance of a significant decrease in blood sugar which can trigger hypoglycemia (Sangkal, 2021).

4. CLOSING

Based on the results of the study of the antidiabetic activity of the ethyl acetate fraction of takokak fruit, it can be concluded that takokak fruit has antidiabetic activity. The compounds thought to have antidiabetic activity are flavonoids, tannins, and steroids.

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