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Activity Test of N-Hexane Fraction of Takokak Fruit (*Solanum torvum Swartz*) Using Glucose Tolerance Test Method Per Oral (OGTT) in Test Animals White Rats *(Rattus norvegicus)*

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Abstract *Takokak fruit contains bioactive compounds that can be used as antidiabetics. This study aims to identify bioactive compounds of ethanol extract of takokak fruit and antidiabetic test of n-Hexane fraction. The method used to obtain the extract is maceration using ethanol . Antidiabetic testing is carried out through in vivo testing with the Oral Glucose Tolerance Test (OGTT) method on white rats. Secondary metabolites are compounds contained in taakokak fruit that are identified as flavonoids, saponins, tannins, steroids and terpenoids . The results of the antidiabetic test showed that the n-Hexane fraction with glibencamide as a comparator has the ability as an antidiabetic in terms of the amount of difference in blood glucose levels in the test animal group from* T_{30} *to* T_{120} *. The average decrease in blood glucose levels in the negative control group (Na-CMC) was 108.67 mg/dL, the positive control group (glibencamide) 174.67 mg/dL, the 5% concentration fraction group 35.33 mg/dL, the 10% concentration fraction group 30.67 mg/dL, the 20% concentration fraction group 47.67 mg/dL.*

Keywords *: Takokak Fruit, Bioactive Compounds, Antidiabetic, TTGO*

1. INTRODUCTION

Diabetes is a non-communicable disease, but its prevalence continues to increase every year. The latest estimate from *the International Diabetes Federation* (IDF *)* , there were 382 million people living with diabetes in the world in 2013. This number is expected to increase to 592 million people in 2035. The use of safe antidiabetic drugs that do not have many side effects is very necessary, considering that the treatment is long-term. Currently, diabetes treatment uses a lot of synthetic drugs that can cause permanent organ damage. Medical diabetes therapy also requires relatively expensive costs. These two factors trigger the high mortality rate of sufferers. This makes people start to switch to alternative or traditional medicine by consuming medicinal plants that tend to be easy to obtain and have low side effects (Mahargyani, 2019) . Antidiabetic is an activity given by certain compounds that can treat diabetes. Testing for antidiabetic activity is usually done on herbal plants . This test is carried out with three methods, namely *in vitro, in vivo and in silico* (Nugraha, 2018). The Oral Glucose Tolerance Test (OGTT) is a glucose tolerance test, where this method test is a test carried out *in vivo* on test animals. 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 by using samples from natural materials, namely takokak fruit, which is extracted after which fractionation is carried out .

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Fractionation is a process for extracting compounds from an extract using 2 types of solvents that do not mix with each other. Solvents that are generally used for fractionation are n-Hexane, ethyl acetate, and methanol, to extract fat and non-polar compounds using n-Hexane, ethyl acetate to extract semi-polar compounds, while methanol to extract polar compounds (Irwan, 2017).

Takokak fruit (*Solanum torvum Swartz*) is a plant that has antidiabetic activity. Apart from being consumed as a side dish, Indonesian people often use takokak fruit as a traditional medicine. for diabetes mellitus (Hidayat, 2015) . This is related to the active compounds contained in it, namely *glucoalkaloids, solasonin, sterolin* , protein, fat, and minerals. Takokak fruit contains various types of vitamins, such as vitamin A, vitamin B1, and vitamin C. The presence of bioactive compounds causes takokak fruit to be used as an antioxidant, cardiovascular, antimicrobial, and antidiabetic.

The plant (*Solanum torvum Swartz*) originates from South America and has spread widely in throughout Asia. The plant grows in forests, riverbanks, fields, gardens and is also cultivated in home page. Takokak plant (*Solanum torvum Swartz*) is generally used as a vegetable and has medicinal properties, to improve blood circulation, relieve pain (analgesic) and relieve cough (antitussive) .

2. METHOD

Tools and materials

The equipment that will be used is an oral probe, syringe, filter paper, funnel, flannel, glass container, blood sugar strips, and glucometer, analytical scales, *hot plate* , *water bath, aluminum foil* , animal cages, food and drink containers, and gloves. While the materials used in this study are Takokak fruit extract, distilled water, glucose, 0.5% Na CMC, n-Hexane, hydrochloric acid (HCl), chloroform ($CHCl_3$) Magnesium powder (Mg), sulfuric acid (H_2SO_4), Liebermann-burchard, Mayer's reagent, Dragendroff's reagent, Wagner's reagent, $FeCl_3$ and chloroform solvent, acetone, 95% ethanol.

Research Procedure

The samples used in this study were taken from Bengkol, Mapanget District, Manado City. The sample was weighed as much as 800 grams, put into a container, extracted by maceration for one week using 95% ethanol. The macerate obtained was separated from the solvent using a rotary evaporator to obtain a thick extract. The extract obtained was divided into two parts for testing purposes, namely phytochemical screening and n-Hexane

fractionation. The fractionated filtrate obtained was evaporated to obtain a thick extract which was then tested for antidiabetic properties.

Terpenoid Test

Terpenoid test is done by taking a sample of 2 mL and putting it into a test tube, then adding 0.5 mL of acetic acid and then adding 2 mL of Lieberman-Buchard through the wall of the test tube. Positive test results are indicated by a change in color or the formation of a brownish ring (Latifah, 2015).

Fractionation

Ethanol extract 95% was dissolved in water and stirred until all the extract dissolved, mix the extract and water into a separating funnel and fractionation was carried out using n-hexane solvent as much as 900 mL and 100 mL of distilled water or a ratio of 1: 9. The results of the fraction were stored and the residue obtained was added with distilled water, the mixture was shaken and left until 2 layers were formed, namely the water layer and the n-Hexane layer. The two layers were separated, then the n-Hexane fraction obtained was evaporated with *a rotary evaporator* . The n-Hexane fraction obtained was weighed and its yield value was calculated (Hasanah, 2019) .

Antidiabetes Activity Using The Oral Glucose Tolerance Method (OGTT)

The initial stage of the test animals was fed and watered regularly for 1 week, fasted for 12 hours (still given water). The test animals were weighed to determine their body weight, where the results of the weight measurement will be used in administering the dose of the test material. The second stage, initial blood sugar levels were measured using a glucometer, blood *test strips* through the tail vein of rats 0 (T_0) . After that, the test material was given according to the dose orally. The third stage, at the 30th minute (T_{30}) of the administration of the test material, glucose was given according to the dose orally. The fourth stage was measuring blood sugar levels with time intervals of 60 minutes (T_{60}), 90 minutes (T_{90}), 120 minutes (T_{120}) (Sangkal, 2021) .

3. RESULTS AND DISCUSSION

The extraction results from 800 grams of takokak fruit *(Solannum torvum Swartz)* obtained a thick extract of 49.73 grams and were divided into two parts for phytochemical screening and fractionation. Based on the results of phytochemical screening examination of 95% ethanol extract of takokak fruit, there were chemical compounds of the flavonoid, tannin, saponin, terpenoid and steroid groups. The results of phytochemical screening can be seen in table 1.

Identification of *flavonoid compounds* in takokak fruit extract was carried out by reacting the sample with Magnesium powder and concentrated HCl into 2 mL of takokak fruit extract in a test tube, the color change that occurred was orange or brick red. This indicates that the takokak fruit extract positively contains flavonoid compounds . *Flavonoids are a group of polar phenol* compounds *found* in almost *every* plant. The addition of magnesium powder and HCl to the *ethanol extract* of takokak fruit can cause the *flavonoid compounds* to be reduced, resulting in a change in the color of the extract solution to orange/brick red (Pujiwidodo, 2016) . The results of the identification of *flavonoid* compounds in takokak fruit extract can be seen in Figure 1.

Figure 1. Results of compound identification Identification of *flavonoid compounds tannin* compounds in takokak fruit extract using FeCl $_{3}$ shows the presence of *phenol* group content indicated by the formation of a blackish blue color after FeCl $_{3}$ was added . This indicates that the takokak fruit extract positively contains *tannin compounds* . The formation of a blackish blue color in the takokak fruit extract after FeCl $_{3}$ was added because *tannin* will form a complex compound with Fe $^{3+}$ ions (Marpaung *et al* ., 2017) . The results of the identification of tannin compounds in takokak fruit extract can be seen in Figure 2

Figure 2. Results of identification of *tannin* compounds in takokak fruit extract in figure 3

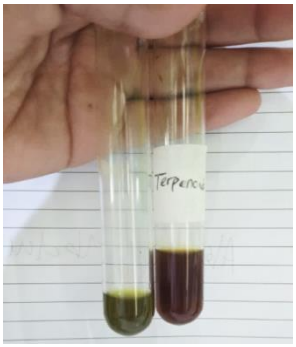

Figure 3. Results of identification *of terpenoid* compounds in takokak fruit extract.

Fractionation Results

The thick extract of takokak fruit as much as 43.73 grams obtained from the extraction results was fractionated using n-Hexane solvent and distilled water. Based on Sutrisno's research (2016) n-Hexane solvent is used for fractionation because it is a non-polar solvent while distilled water is a polar solvent, in accordance with the purpose of fractionation, namely separating compounds based on solubility in solvents with different levels of polarity. So that non-polar compounds can be attracted by non-polar solvents or n-Hexane and distilled water to attract polar compounds. During fractionation, 2 layers are formed. The first layer is the remainder or residue from the fractionation process, the second layer is the n-Hexane fraction. The first fraction is separated from the second fraction, then the n-Hexane fraction is thickened. The n-Hexane fraction of the ethanol extract of takokak fruit produced was 18.4 grams and the yield was 42.07%. The results of fractionation can be seen in Figure 4

Figure 4. Results of n-Hexane fraction

concentration of 20% blood sugar levels at T_{30} to T_{60} decreased by 23.34 mg/dL, at T_{60} to T_{90} decreased by 9.33 mg/dL and at T_{90} to T_{120} decreased by 20.33 mg/dL. The difference in the decrease in blood glucose levels for each test group can be seen in Figure 5

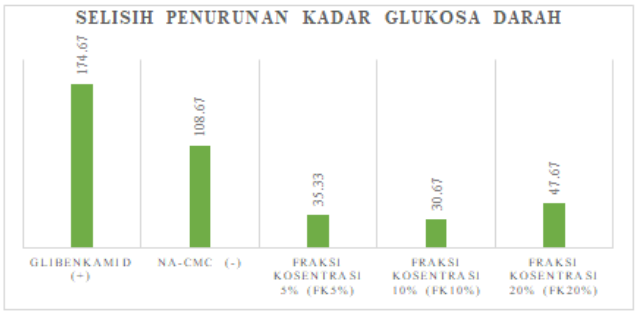

Figure 5. Difference in the decrease in blood glucose levels of the test group from T_{120} to T_{30}.

Judging from the difference in the decrease in glucose levels from T_{120} to T_{30}, each treatment group experienced a decrease in blood glucose levels of (-) 174.67 mg/dL for the positive control group, (-) 108.67 mg/dL for the negative control group, (-) 35.33 mg/dL for the 5% concentration fraction group, (-) 30.67 mg/dL for the 10% concentration fraction group, (-) 47.67 for the 20% concentration fraction group. These results indicate that takokak fruit extract and its fractions with glibenclamide as a comparator have the potential to reduce blood glucose levels in test animals. The decrease in blood sugar levels in the glibenclamide test group in this case used as a comparator had the largest difference compared to other test groups. The magnitude of this difference is influenced by time where the longer the time the decrease in blood sugar levels is, the greater it is so that if it is not controlled, there is a possibility that there will be a significant decrease in blood sugar levels and result in *hypoglycemia.* (Sangkal, 2021) . The compound that is thought to play a major role in the antidiabetic activity of takokak fruit is *methyl caffeate* . *Methyl caffeate* is included in the *flavonoid compounds* that can reduce blood glucose concentration and increase insulin (Silalahi, 2019) .*Thesis.* Bogor Agricultural University.

Phytochemical Screening of Takokak Fruit Extract

Alkaloid Test

Alkaloid test is done by taking a sample of 2 mL and putting it into a test tube, then adding 2 mL of ammonia and chloroform then adding 3-5 drops of $_{\text{concentrated}}$ H_2SO_4 $_{\text{then}}$ shaking until two layers are formed, then transfer the top layer into three different test tubes, each 2.5 mL. Then the three solutions are added with 4-5 drops of Wagner, Mayer and Dragendorf reagents. Samples that are positive for containing alkaloid compounds will form a white precipitate from the Mayer reagent, a red-orange precipitate from the Dragendorf reagent and with Wagner reagent it produces a brown precipitate (Mondog *et al* ., 2015).

Flavonoid Test

Flavonoid test is done by taking a sample of 2 mL, put it into a test tube then add 2 mL of ethanol then heated until boiling then filtered, then added 0.05g of Mg powder and 1 mL of concentrated HCL then shaken. Positive test results are indicated by the formation of orange-yellow to red colors (Mondog *et al* ., 2015).

Tannin Test

The tannin test is carried out by taking a sample of 2 mL and then adding a few drops of FeCl3 . A positive test result will produce a green or blackish blue color (Ergina, 2014).

Saponin Test

The saponin test is carried out by inserting 2 mL of sample into a test tube then adding 10 mL of distilled water then shaking for 10 seconds and observing the changes that occur. If a stable foam of 1-10 cm is formed for no less than 10 minutes, it is positive for containing saponins (Mutmainnah, 2017).

Steroid Test

Steroid test is done by taking 2 mL of each sample and then adding 3 drops of concentrated HCl and 1 drop of Lieberman-Buchard. Positive results are indicated by the formation of a green solution (Ergina, 2014).

Table 1. Results of phytochemical screening of ethanol extract of takokak fruit.

Senyawa	Pereaksi	Pengamatan	Hasil Pengamatan	Literatur
Alkaloid	Amonia + Kloroform + H_2SO_4 + Pereaksi Mayer	Tidak ada endapan	(-)	Endapan putih (Mondong *et al.*, 2015)
	Amonia + Kloroform + H_2SO_4 + Pereaksi Wagner	Tidak ada endapan	(-)	Endapan merah jingga (Mondong *et al.*, 2015)
	Amonia + Kloroform + H_2SO_4 + Pereaksi Dragendrof	Tidak ada endapan	(-)	Endapan coklat (Mondong *et al.*, 2015).
Flavonoid	Serbuk Magnesium + HCl Pekat	Terjadi pembentukan warna jingga	(+)	Menghasilkan warna kuning, jingga hingga merah (Mondong *et al.*, 2015).
Saponin	Aquadest	Berbusa stabil	(+)	Berbusa stabil tidak kurang dari 10 menit (Mutmainnah, 2017).
Tanin	$FeCl_3$ 10%	Terjadinya pembentukan warna biru kehitaman	(+)	Menghasilkan warna hijau atau biru kehitaman (Ergina,2014)
Steroid	HCl Pekat + Lieberman Buchard	Terjadinya pembentukan warna hijau	(+)	Menghasilkan warna hijau (Ergina, 2014)
Terpenoid	CH_3COOH + Lieberman Buchard	Trebentuknya cincin kecoklatan	(+)	Terbentuknya cincin kecoklatan (Latifah, 2015).

Keterangan : (-) negatif ; (+) positif

Based on table 1, the identification of alkaloid compounds in takokak fruit extract showed negative results, which were indicated by the absence of sediment formation in the extract added with Mayer, Wagner and Dragendorff reagents. Takokak fruit extract in this study did not contain alkaloid compounds. Based on research conducted by Hidayati 2015

entitled " The Effect of Boiling on Biological Activity and Phenolic Content of Takokak Fruit *(Solanum Torvum Swartz)* " that the ethanol extract of takokak fruit did not contain *alkaloid compounds* . *Alkaloids* are compounds that are basic. *Alkaloid testing* using Mayer, Wagner and Dragendorff reagents did not produce sediment formed from ligand replacement (Simare, 2014) . The sediment is formed because the nitrogen atom that has a free electron pair in the alkaloid replaces the iodine ion in the Mayer, Wagner and Dragendorf reagents through covalent bonds. If no sediment is formed, it can be concluded that the extract does not contain alkaloid compounds. The results of the identification of alkaloid compounds in takokak fruit extract can be seen in Figure 6.

Figure 6. Results of identification of *alkaloid* compounds in takokak fruit extract.

Takokak fruit extract was detected positive for containing saponins which was indicated by the appearance of stable foam or foam for less than 10 minutes. The addition of distilled water in the *saponin test* caused an increase in the polarity of the *saponin compound* so that there was a change in the polarity of its composition. In this condition, the polar (*hydrophilic*) group will face outward and the non-polar (*hydrophobic*) group will face inward and form a structure called *micelles* . This condition forms foam which is a sign of the presence of saponin compounds in the extract (Pujiwidodo, 2016) . The results of the identification of saponin compounds in takokak fruit extract can be seen in Figure 7.

Figure 7 Results of identification of *saponin* compounds in takokak fruit extract.

Identification of steroid compounds from *ethanol extract* of takokak fruit obtained results that there was a green color change which means that the takokak fruit extract positively contains *steroid compounds* . The color change is due to oxidation in the steroid compound

group through the formation of conjugated double bonds (Sangkal, 2021) . The results of the identification of *steroid compounds* from takokak fruit extract can be seen in Figure 6.

Figure 8. Results of identification of *steroid* compounds in takokak fruit extract.

terpenoid compounds in *ethanol* extract of takokak fruit showed positive results which were marked by the formation of a brown ring. The use of *ethanol* in the extract can identify the presence of *terpenoid compounds. The polarity level of ethanol* solvents is higher than that of *methanol solvents* . This is because *terpenoid compounds* have polar -OH groups, so they can be extracted with polar solvents as well (Bhernama, 2020) . The results of the identification of *terpenoid compounds in* takokak fruit extract can be seen

Antidiabetic Test Results

Antidiabetic tests were conducted on white rats *(Rattus norvegicus)* using the n-Hexane fraction of takokak fruit with an oral glucose tolerance test (OGTT). The test results were obtained from measuring the average blood sugar levels of the test animal group with a time interval of T_0 to T_{120}. Based on the results of measuring the average blood glucose levels of the test animals before and after receiving treatment. At T_0, the blood glucose levels of all treatment groups were in the normal category based on the normal blood sugar levels of the test animals, which were 75-150 mg/dL (Samsuri *et al* ., 2020) . It can be seen that at T_{30} the blood glucose levels for the positive control group were (+) 127 mg/dL, for the negative control group (+) 3.33 mg/dL, for the FK5% group (+) 83.33 mg/dL, for the FK10% group (+) 77.67 mg/dL, and for the FK20% group (+) 27 mg/dL as in table 2.

Kelompok perlakuan	Rata-rata kadar gula darah				
	T_0	T_{30}	T_{60}	T_{90}	T_{120}
Glibenkamid (+)	91,33 mg/dL	295 mg/dL	52,33 mg/dL	106 mg/dL	120,33 mg/dL
Na-CMC (-)	106 mg/dL	233 mg/dL	102,66 mg/dL	93 mg/dL	124,33 mg/dL
Fraksi kosentrasi 5% (FK5%)	100,33 mg/dL	183,66 mg/dL	64,33 mg/dL	174,33 mg/dL	148,33 mg/dL
Fraksi kosentrasi 10% (FK10%)	77,33 mg/dL	155 mg/dL	124,33 mg/dL	142 mg/dL	124,33 mg/dL
Fraksi kosentrasi 20% (FK20%)	86 mg/dL	113 mg/dL	89,66 mg/dL	80,33 mg/dL	65,33 mg/dL

Based on table 2, it can be seen that at T_{30} all treatment groups were given glucose solution. At T_{60} to $T_{120,}$ there was a decrease in blood glucose levels where the results of the average measurement of blood sugar levels in the test group from T_{30} to T_{120} for the positive control test group, blood sugar levels at T_{30} to T_{60} decreased by 242.67 mg /dL, at T_{60} to T_{90} decreased by 53.67 mg/dL and at T_{90} to T_{120} decreased by 14.33 mg/dL. For the negative control test group, blood sugar levels at T_{30} to T_{60} decreased by 130.34 mg/dL, at T_{60} to T_{90} decreased by 9.66 mg/dL and at T_{90} to T_{120} decreased by 31.33 mg/dL. For the test group of n-Hexane fraction of takokak fruit with a concentration of 5%, blood sugar levels at T_{30} to T_{60} decreased by 119.33 mg/dL, at T_{60} to T_{90} decreased by 110 mg/dL and at T_{90} to T_{120} decreased by 26 mg/dL. For the test group of n-Hexane fraction of takokak fruit with a concentration of 10%, blood sugar levels at T_{30} to T_{60} decreased by 30.67 mg/dL, at T_{60} to T_{90} decreased by 110 mg/dL and at T_{90} to T_{120} decreased by 17.67 mg/dL. For the test group of n-Hexane fraction of takokak fruit

Gandhi *et al* (2011) stated that methyl *caffeate causes increased insulin secretion from* β cells , therefore the effect of methyl *caffeate* is almost the same as *glibencamide* which stimulates insulin secretion from β cells and reduces blood glucose levels in diabetes.

4. CONCLUSION AND SUGGESTIONS

Based on the results of the study of the antidiabetic activity test of the n-Hexane fraction of the takokak fruit *(Solanum torvum swartz)* using the Oral Glucose Tolerance Test (OGTT) method on white rats *(Rattus norvegicus),* it can be concluded that the ethanol extract of the takokak fruit contains *flavonoids, tannins, saponins, steroids* and *terpenoids* . The n-Hexane fraction of the takokak fruit can lower blood glucose levels so that it can be used as an antidiabetic treatment. The suggestion for further research is that the research period should be extended to see the effect of lowering blood sugar levels significantly and isolation is carried out so that it can be used as an alternative treatment that is useful for the community. It is hoped that institutions can replace expired laboratory materials in order to obtain more effective research results.

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