Indonesian Journal of Global Health Research
Volume 6 Number 3, Juni 2024
e-ISSN 2715-1972; p-ISSN 2714-9749
http://jurnal.globalhealthsciencegroup.com/index.php/IJGHR

TEST FOR INHIBITION OF THE ENZYME α-GLUCOSIDASE FRACTION OF ETHANOL EXTRACT OF ONCO LEAVES (Spondias pinnata (L.f.) Kurz.) AND THE ANTIDIABETIC ACTIVITY OF THE SELECTED FRACTION

Fajria Rezky Priyanti Amara Pagal*, Titik Sunarni, Supriyadi
Master of Pharmacy Study Program, Faculty of Pharmacy, Universitas Setia Budi Surakarta, Jl. Letjen Sutoyo, Mojosongo, Jebres, Surakarta, Central Java 57127, Indonesia
*fajriarezkyamarapagal@gmail.com

ABSTRACT

Degenerative diseases are the number one disease in Southeast Asia. Based on WHO data in 2008, the death rate in Southeast Asia was around 14.5 million, around 55% (7.9 million) caused by degenerative diseases. One of the degenerative diseases is diabetes mellitus. Diabetes mellitus is a metabolic disorder characterized by hyperglycemy. Research that has been carried out to look at the antidiabetic activity of onco (Spondias pinnata (L.f.) Kurz) leaves is still limited to the extraction results. Objectives: This study aims to determine the fraction of onco leaf ethanol extract in inhibiting the enzyme *alpha glucosidase*, having antidiabetic activity and histopathological picture of pancreatic cells in streptozotocin-induced male white rats (*Rattus norvegicus*). This study used inhibition testing of the enzyme α-glucosidase fraction of onco leaf ethanol extract with concentrations of 12.5, 25, 50,100 and 200 µg/mL using a *microplate reader* with a wavelength of 405 nm, as well as with experimental laboratory test animal methods as many as 28 mice divided into 7 treatment groups the induced streptozotocin except the normal group. Group I as normal control of rats that were only given regular drinking food, group II as negative control without treatment, group III as positive control using glibenclamide drugs and group IV ethanol extract 500, V n-hexane fraction 132.54, VI ethyl acetate fraction 126.24 and VII water fraction 239 mg/KgBB treatment given for 14 days. The results showed that onco leaf ethanol extract inhibits the enzyme α-glucosidase with very strong strength (IC50 = 39.13 µg / ml) and the n-hexane fraction has the effect of reducing glucose levels as well as the ability to correct the histopathology of the pancreas of male white rats (*Rattus norvegicus*) induced streptozotocin. Conclusions: Ethanol extract and the fraction of onco leaves (*Spondias pinnata* (L.f.) Kurz) has antidiabetic activity with a mechanism of action inhibiting the enzyme *alpha glucosidase* and the activity of decreasing blood glucose levels and has the ability to correct the histopathology of the pancreas of male white rats (*Rattus norvegicus*) induced streptozotocin.

Keywords: antidiabetic; onco leaf; rat pancreatic histopathology

First Received	Revised	Accepted
04 April 2024	08 April 2024	09 April 2024
Final Proof Received		**Published**
13 April 2024		01 June 2024

How to cite (in APA style)
Pagal, F. R. P. A., Sunarni, T., & Supriyadi, S. (2024). Test for Inhibition of the Enzyme A-Glucosidase Fraction of Ethanol Extract of Onco Leaves (Spondias pinnata (L.f.) Kurz.) and the Antidiabetic Activity of the Selected Fraction. Indonesian Journal of Global Health Research, 6(3), 1433-1444. https://doi.org/10.37287/ijghr.v6i3.3166.

INTRODUCTION

Diabetes mellitus is a metabolic disorder syndrome characterized by abnormal hyperglycemia as a result of a deficiency in insulin secretion, reduced activity of the biological function of insulin or the presence of insulin resistance and then the β cells show disturbances in the first phase of insulin secretion, meaning that insulin secretion fails to compensate for insulin resistance (Khalid et al., 2022). (type 2 DM) or absolute insulin deficiency (type 1 DM). Symptoms and signs of hyperglycemia such as polyuria, polydipsia, weight loss are often accompanied by chronic symptoms (Tandi & Muthi'ah, 2016).

Most diabetes mellitus sufferers use synthetic drugs to lower blood glucose levels (Salehi et al., 2019). However, these drugs have side effects such as hypoglycemia, weight loss and diarrhea so other treatments are needed such as using traditional medicines (Mohamed et al., 2015). As is known, Indonesia has a diversity of plants, especially world-famous medicinal plants which have antioxidant and anti-diabetic effects. One of them is the onco plant (*Spondias pinnata* (Lf) Kurz). Previous research conducted by Sai et al (2021) explained that the onco leaf plant (*Spondias pinnata* (Lf) Kurz) contains secondary metabolic compounds, namely alkaloids, saponins and flavonoids which are antioxidants and can reduce blood glucose levels in mice with an extract dose of 500 mg/Kg BW. Apart from that, research by Preeti Jain et al (2014) stated that onco leaf extract has antioxidant potential. The extract was evaluated using several in vitro models including 1,1-*diphenyl-2-picrylhydrazyl* (DPPH), *nitric oxide*, and *superoxide radical scavenging assays and reducing power estimation*. The highest flavonoids were shown by ethyl acetate extract (extract 86.53 ± 1.95 mg QE/g).

Research that has been carried out to look at the antidiabetic activity of onco (*Spondias pinnata* (Lf) Kurz) leaves is still limited to the extraction results, whereas in this study, fractions from onco (*Spondias pinnata* (Lf) Kurz) leaves were used using solvents with different polarity levels, namely n -hexane, ethyl acetate and water. This was done to determine the active group that produces antidiabetic activity on the histopathological picture of the pancreas in STZ-induced mice. This antidiabetic effect is expressed as a decrease in blood glucose levels and an improvement in the histopathological profile of the rat pancreas. In addition, onco (*Spondias pinnata* (Lf) Kurz) leaves can be seen as antidiabetic using in vitro inhibition of the α-Glucosidase enzyme. This study aims to analyze the α-glucosidase enzyme inhibition test of the ethanol extract fraction of onco leaves (*Spondias pinnata* (l.f.) kurz.) and the antidiabetic activity of selected fractions.

METHOD

This research used a laboratory experimental method with a modified pre & post test randomized controlled group design, consisting of two treatment groups, namely the control group (group I: normal control, group II: negative control and group III: comparison control) and the experimental group (groups IV, V, VI and VII), where the experimental group was given treatment while the control group was not given treatment. The subject of this research is onco leaf simplicia (Spondias pinnata (L.f.) Kurz) obtained in the Poso area, Central Sulawesi. This research was carried out at Universitas Setia Budi, Surakarta in October-January 2022.

Material

The materials used in this research were onco leaf simplicia (*Spondias pinnata* (Lf) Kurz) obtained from the Poso city area, Poso Regency, Tegah Sulawesi, 96% ethanol (Brataco) as a solvent, ethyl acetate, n-hexane, chloroform, Mayer's reagent, Anisaldehyde, FeCl3, distilled water, NaCMC, glibenclamide, streptozotocin, α-glucosidase enzyme assay kit, dragendrof LP, ether, 10% formalin, handskun, cotton, label paper, filter paper, Mayer hematoxylin-eosin solution, mask, sodium hydroxide, sodium chloride, standard feed, streptozotocin.

Preparation of extract

Simplicia powder was extracted by maceration using 96% ethanol solvent. 500 grams of Simplicia powder was weighed then put into a maceration vessel using 5 L of ethanol solvent, covered, then left for 3x24 hours protected from light, stirring occasionally. The extract was filtered using filter paper, then the filtrate was obtained, concentrated using a rotary evaporator at a temperature of 70oC and continued with thickening using a water bath at a

temperature of 60oC until it became a thick extract. The results of the concentration were then tested for ethanol free and the extract yield was calculated.

Fraction creation

Making fractions from onco leaf extract involves taking the leaf extract and then partitioning it successively using the solvents n-hexane, ethyl acetate and water. Weigh 20 grams of the thick extract, dissolve it in 40 ml of distilled water, then put it in a separating funnel and add 40 ml of n-hexane, shake it then let it sit until 2 layers are formed, then separate them. The water fraction was extracted liquid-liquid with the addition of 40 ml of ethyl acetate, shaken and then allowed to stand until 2 layers were formed, the ethyl acetate layer (bottom layer) was separated. Then the fraction obtained was evaporated using a *rotary evaporator* until thick. Najihudin et al., (2017) and used as a test fraction in STZ-induced DM rats.

Phytochemical test

Phytochemical tests aim to provide an initial picture of the content of certain compounds in the natural ingredients being studied (Vifta, 2018).

Organoleptic test

Organoleptic testing includes observing the shape, color, smell and taste to physically determine a product (Aguila-Muñoz et al., 2023).

Alkaloid examination

The sample was spotted on a GF254 TLC plate, put into a saturated vessel containing the mobile phase toluene: ethyl acetate: diethylamine (7:2:1) eluted to the limit, removed and dried, then sprayed with Dragendroff's reagent. Alkaloid compounds using dragendroff give an orange or brown color after being heated for 5-10 minutes at 100°C (Wegner and Bladt, 1995). The results of the analysis show orange or brown spots (Daou et al., 2022).

Saponin examination

The sample was spotted on a GF254 TLC plate, placed in a saturated vessel containing the mobile phase chloroform: methanol: water (6:3:1). The appearance of the stain can be seen at UV 254 nm, 366 nm is green. Anisaldehyde spray reagent produces a purple color and appears blue under light spots (Niaz et al., 2021).

Tannin examination

The sample was spotted on a GF254 TLC plate, put into a saturated vessel containing the mobile phase acetic acid: formaldehyde: acetic acid: water (10:11:11:27), then put in the chamber, eluted to the mark. With FeCl3 reagent staining. A positive reaction is indicated by the formation of a violet green stain (Dona et al., 2021).

Inspectionflavonoids

The sample was spotted on a GF254 TLC plate, the plate was put into a saturated vessel containing the mobile phase hexane: ethyl acetate: formic acid (6: 4:2) then eluted to the mark. The plate was dried and observed under UV light. Detection of flavonoid compounds was carried out using cytroborate reagent, after heating for 5 minutes at 100°C, yellow, greenish fluorescence will occur at UV 366 nm (Yuriah et al., 2023)

Inhibition test of α-glucosidase enzyme activity in onco leaves

Test the inhibitory activity of the α-glucosidase enzyme using 20 µL of α-glucosidase and 120 µL of 0.1 M phosphate buffer. As a substrate, 5 mM p-nitrophenyl-α-D glucopyranoside (p-

NPG) was used in the same buffer. A total of 10 μL of the test extract which had been dissolved with DMSO in various concentrations, was mixed with the enzyme solution in wells (96-well microplates) and incubated at 37°C for 15 minutes (Mohamed et al., 2015). During preparation and testing, the enzyme must be specially treated, the enzyme solution must be stored at a temperature of 2-80 C (Sigma, 1996). Then 20 μL of substrate solution was added and incubated again at 37°C for 15 minutes. The enzymatic reaction was stopped by adding 80 μL of 0.2 M sodium carbonate solution. The reaction system without extract was used as a control and blank, while the system with extract was used as a test sample. The test was carried out three times. The absorbance of the samples was measured using a microplate reader at a wavelength of 405 nm.

Preparation of test animals

Twenty-eight male white rats were divided into 7 groups and adapted for 1 week in the laboratory and given standard feed and their initial glucose levels were measured as T0, then induced with streptozotocin except group 1. After 3 days of streptozotocin induction, their blood glucose levels were measured as T1. Mice with blood glucose levels greater than 200 mg/dl were given further treatment.

How to draw blood

Blood samples were taken from each rat from the tail vein and their blood glucose levels were measured using a glucometer to ensure that all Wistar rats had normal blood glucose levels before being given treatment. Normal blood glucose levels in mice range between 50-135 mg/dl. Before use, the glucometer is turned on and the glucose stick is inserted into the glucometer. Blood was taken from the tip of the rat's tail which had previously been cleaned with 70% alcohol, then sorted slowly and then the tip of the tail was pierced with a small needle. The blood that comes out is then dripped onto the glucometer stick, within 10 seconds the blood glucose level will be measured automatically and the results can be read on the glucometer monitor.

Pancreatic histopathological testing

Pancreatic histopathological testing was carried out after treatment on the 28th day. The test animals were sacrificed using anesthesia, that is, the mice were placed in a jar containing cotton wool treated with ether. Wait until the rat loses consciousness by giving painful stimuli to the soles of the rat's feet, if it doesn't respond then the anesthetic effect has worked. The surgical process was carried out on the skin of the stomach until the internal organs of the rat's stomach were visible. The pancreatic organ was taken and placed in a special container containing formalin 10 (Prameswari, 2013). The first thing that is done is to remove the organ by sacrificing the test animal under anesthesia, then the entire pancreas is taken. Then the pancreatic tissue was made for histopathological preparations.

RESULTS

Results of making ethanol extract of onco leaves

Making 500 grams of onco leaf ethanol extract using the maceration method. The solvent used was 96% ethanol. done by maceration. The maceration process was carried out for three days with occasional shaking using 5 L (500:5 L) of 96% ethanol, then the extraction results were concentrated using a Vacuum Rotary Evaporator at a temperature of 50°C. Obtained thick extract and percent yield of onco leaf extract were 56.72 g and 11.344% respectively.

Results of making fractions from ethanol extract of onco leaves

Making fractions from onco leaf extract using the liquid-liquid extraction method, namely taking the leaf extract and then partitioning it successively using the solvents n-hexane, ethyl acetate and water. It was found that the weights of the n-hexane, ethyl acetate and water fractions were 4.32, 4.05 and 7.67 g respectively and the yield percentages were 21.6,20.25 and 38.35% respectively.

Results of organoleptic observations of onco leaves

Organoleptic tests are carried out by observing the shape, color, odor and taste of the extract. On organoleptic examination, the extract has a thick shape, dark green color, has a distinctive smell and a bitter taste.

Results of examining the chemical content of onco leaf extract fractions using TLC

The identification test used the TLC method with the stationary phase silica gel F254, and the mobile phase using several solvents. The purpose of choosing a solvent as the mobile phase is because each solvent has a different polarity so that compounds with different polarities are expected to be separated from the eluent. The results are seen after the TLC plate is sprayed with a reagent to reveal spots or stains under UV 366.

Table 1.
Results of Chemical Content Examination by TLC

Compound	Reactor	Results			
		Extract	N-Hexane	Ethyl acetate	Water
Alkaloids	Dragendroff	+	+	+	-
Saponins	Anisaldehyde	+	+	+	+
Flavonoids	Sitroborat	+	-	+	+
Tannin	FeCl3	+	-	+	+

Based on the results of examination of the content of alkaloid compounds found in the ethanol extract, n-hexane fraction and ethyl acetate of onco leaves, saponin compounds were found in the ethanol extract and all fractions of onco leaves, flavonoid and tannin compounds were found in the ethanol extract, ethyl acetate fraction and water. This content is an antioxidant which is known to prevent damage to pancreatic β cells because it has activity by capturing or neutralizing free radicals associated with phenolic OH groups so that it can repair the condition of damaged tissue (Yuriah, 2024), and is able to reduce oxidative stress by preventing chain reactions changing superoxide. into hydrogen superoxide by donating a hydrogen atom from the aromatichydroxyl (-OH) group to bind free radicals and eliminate them from the body through excretion

Results of the α-Glucosidase Enzyme Inhibition Test

Enzyme inhibition testing was based on the absorbance value of p-nitrophenol with yellow color intensity using a microplate reader (ELISA reader spectrophotometry) (ƛ = 405). The α-glucosidase enzyme hydrolyzes p-nitrophenyl-α-D-glucopyranoside (P-NPG) into glucose and p-nitrophenol which are yellow. However, with the presence of an α-glucosidase (IAG) enzyme inhibitor, the resulting yellow color has varying intensities according to its inhibitory power. The lower the intensity of the yellow color of p-nitrophenol, the less glucose product is produced (Lorenza, 2012).

Table 2.
Results of Inhibition of the A-Glucosidase Enzyme on the Ethanol Extract Fraction

Sample	Mean IC50 ± SD	Category
Acarbose	47.17 ± 2.43	Very strong
N-Hexane	66.06 ± 2.46	Strong
Ethyl acetate	56.36 ± 3.75	Strong
Water	51.47 ± 1.99	Strong
Extract	39.13 ± 2.62	Very strong

Blood Glucose Level Measurement Results

Blood glucose levels were measured 5 times, where 28 male white mice were divided into 7 groups and adapted for 1 week in the laboratory and given standard feed. and the initial glucose level was measured as T0, then induced with streptozotocin except the normal group. After 3 days of streptozotocin induction, blood glucose levels were measured as T1. Mice with blood glucose levels greater than 200 mg/dl were given further treatment. All groups were given treatment according to their respective groups for 3 weeks. Blood sampling was carried out on the 10th day (T2), 17th day (T3), and 24th day (T4) to see a decrease in the mice's blood glucose levels. Then the mice were dissected for pancreatic histopathological testing. The decrease in blood glucose levels can be seen in figures 8 and 9 below.

Table 3.
Average Blood Glucose Levels of Onco T0 and T1 Leaf Extract Rats

Group	Results of glucose levels (mg/dl) ± SD	
	Day 0 (T0)	Day 3 (T1)
Normal Control	87.25 ± 12.04	91.75 ± 12.58
Positive Control	90± 10.12	370.75±13.63
Negative Control	85± 10.46	336.25±66.89
N-Hexane	90± 12.19	398.5± 49.65
Ethyl acetate	88± 9.14	377.25±11.69
Water	82.5± 11.93	409.5± 35.81
Extract	103.25± 17.47	416± 51.40

Information :
T0 = Time of measurement of blood glucose levels before STZ induction
T1 = Time for measuring blood glucose levels after STZ induction

Table 4.
Average Blood Glucose Levels of Rats with Onco T1, T2, T3, and T4 Leaf Extract

Group	Results of glucose levels (mg/dl) ± SD			
	Day 3 (T1)	Day 10 (T2)	Day 17 (T3)	Day 24 (T4)
Normal Control	91.75 ± 12.58	93 ± 10.98	87.75 ± 8.95	86 ± 8.92
Positive Control	370.75±13.63	353± 11.25	173± 1.87	95± 5.87
Negative Control	336.25±66.89	378.75±54.79	382.5± 54.49	384.25± 54.43
N-Hexane	398.5± 49.65 a	351.25±15.02a,b,c	172.25± 4.32 c	97.5± 6.84 c
Ethyl acetate	377.25±11.69 a	331.25±22.49a,b,c	177.5± 10.52 a, b, c	106.75± 4.02 a, b, c
Water	409.5± 35.81 a	381.25±35.52a,b,c	179.75± 8.87 a, b, c	106.25± 6.34 a, b, c
Extract	416± 51.40 a	359.5± 52.83a, b, c	169.75± 4.32 a, b, c	100.5± 6.80 a, b, c

Information :
a = Significantly different from normal controls
b = Significantly different from positive control
c = Significantly different from negative control
T1 = Time for measuring blood glucose levels after STZ induction
T2, T3, & T4 = Time for measuring blood glucose levels after administering onco leaf preparations

Pancreatic Histopathology Results

The histopathological picture of the pancreas was evaluated by observing the condition of the islets at 1000x magnification on each slice of the pancreatic preparation. The parameter looked at is a comparison of the condition of the pancreatic islets in each group. In normal controls, a number of defects can be seen such as pyknosis, karyorrhexis and karyolysis, where pyknosis is characterized by cell shrinkage and increased basophilia, which occurs because DNA is compacted into a solid basophilic mass. Karyolysis is a change due to DNA activity that causes chromatin basophilia to fade. Karyorrhexis is a state of fragmentation in the pyknotic nucleus (St. Aisyah Sijid, 2020). The normal group experienced damage because they physiologically experienced an apoptosis process, where apoptosis is a biological mechanism that experiences programmed cell death which is used to dispose of cells that are no longer used (Nugrahaningsih and Ari Yuniastuti, 2014). Meanwhile, the negative control showed endocrine cell degeneration approaching cell necrosis, as did the treatment group which experienced cell damage as a result of STZ induction. Can be seen in attachment 19.

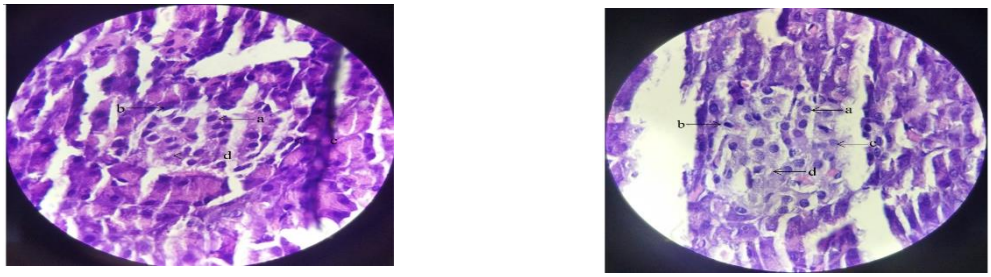

Figure 1. Histopathological image of the pancreas

Information
a: Normal Cells
b: Pycnosis
c: Karyorrhexis
d: Karyolysis

In the negative control that had been induced by STZ, changes were seen, namely endocrine cell degeneration. Degeneration of endocrine cells can be seen in their nuclei which become non-uniform. The nuclei of endocrine cells become smaller (pyknosis) and disappear, so that hyperchromatic cytoplasm is visible. Apart from that, there was also visible clumping of the cytoplasm indicating protein denaturation. This proves that STZ induction can damage pancreatic endocrine cells. STZ is a toxic and selective compound for pancreatic β cells, where it contains glucose molecules so that it can enter the cells through this protein. When the STZ methylnitrosurea molecular group enters the cells, it will produce carbonium ion free radicals (CH3+) which are toxic to DNA so that the nuclei of pancreatic β cells will shrink due to damage, then the cells will experience inflammation and necrosis. When STZ enters cells, it will be metabolized with or without enzymes, producing free radicals, causing oxidative stress in cells because antioxidant defenses are very low. In the negative control that had been induced by STZ, changes were seen, namely endocrine cell degeneration. Degeneration of endocrine cells can be seen in their nuclei which become non-uniform. The nuclei of endocrine cells become smaller (pyknosis) and disappear, so that hyperchromatic cytoplasm is visible. Apart from that, there was also visible clumping of the cytoplasm indicating protein denaturation. This proves that STZ induction can damage pancreatic endocrine cells.

Table 5.
Average Results for the Number of Pancreatic Damage Cells

Group	Mean number of pancreatic damage cells ± SD
Normal Control	22.25 ± 1.48bc
Positive Control	30 ± 1.41 ac
Negative Control	64.5 ± 2.29 ab
N-Hexane	42.5 ± 2.06 abc
Ethyl acetate	49 ± 1.22$^{a\ B\ C}$
Water	52.25 ± 1.92 abc
Extract	44.5 ± 1.12 abc

Information :
a = Significantly different from normal controls
b = Significantly different from positive control
c = Significantly different from negative control

DISCUSSION

The results of calculating the inhibitory activity of the α-glucosidase enzyme show that onco leaf extract has an IC50 value of 39.13 ppm while acarbose has an IC50 value of 47.17 ppm, both of which are in the very strong category and the n-hexane, ethyl acetate and water fractions also have IC50 values. respectively 66.06 ppm, 56.36 ppm and 51.47 are included in the strong category, based on the IC50 results it can be concluded that the extract has an IC50 value in the strong category. This is in accordance with previous research conducted by Fitrilia & M.Bintang (2017) which states that the smaller the IC50 value, the greater its activity in inhibiting the work of the alpha glucosidase enzyme because the ethanol extract of onco leaves has polar properties so that the compounds that can be attracted are still complex where onco leaves contain alkaloids, saponins, tannins and flavonoids so they can inhibit the work of the α enzyme -glucosidase in a competitive or non-competitive manner.

Measurement of blood glucose levels on day 3 (T1) showed that the normal mouse group had no changes compared to several other groups given STZ induction. Blood glucose levels in the group given STZ experienced a significant increase because it was influenced by the test material given to trigger DM conditions, laboratory conditions, living environment and length of treatment can also cause changes in mice's blood glucose levels. Rats that experience stress due to inappropriate drum conditions will affect the measurement of blood glucose levels. Based on the results of measuring blood glucose levels in mice, it shows that giving STZ induction can have a DM effect in test animals. STZ acts as a diabetogenic agent and can damage pancreatic β cells because it has a unique chemical structure and a complex mechanism of action. The mechanism of action of STZ is that STZ works through the methylation pathway, xanthine oxidase and NO production (Alqahtani et al., 2019). These three pathways will produce highly reactive free radicals and disrupt ATP production in mitochondria because they reduce oxygen use by mitochondria and inhibit the Krebs cycle through activation of the PARP-1 enzyme which converts NAD+ into ADP-ribose. As a result, there is a decrease in the amount of ATP and insulin synthesis and secretion. Ultimately, β cell death occurs through the apoptosis mechanism and necrosis will definitely occur (Yuriah et al., 2022).

Days 17 and 24 the treatment group had returned to normal blood glucose levels. This shows that the compounds that are interested in the ethanol extract solvent, n-hexane fraction, ethyl acetate and water are non-polar, semi-polar and polar compounds so that they can reduce blood glucose levels, while for the negative group it shows that there is a significant difference from all treatment, this was because the negative group was not given treatment, it

was only induced by STZ. It is suspected that there are chemical compounds in onco leaves that can work as antihyperglycemia, namely flavonoids, alkaloids, saponins and tannins. These compounds are antioxidant compounds that can reduce blood glucose levels (Rouzbehan et al., 2017). Flavonoid compounds work to inhibit the formation of free radicals which can damage pancreatic β cells by donating hydrogen atoms from the phenolic group to bind with free radicals to form flavonoid radicals and have an inhibitory effect on the alpha glucosidase enzyme by delaying the hydrolysis of carbohydrates and disaccharides and the absorption of glucose and inhibiting metabolism of sucrose into glucose and fructose (Prameswari, 2013). This is in accordance with research conducted by Hazra et al., (2008) onco leaf extract contains the compound quercetin which has an antioxidant effect. Quercetin is a powerful antioxidant,Quercetin is a flavonoid which is included in the flavonol group (Yuriah & Kartini, 2022).

Alkaloids can work by stimulating the hypothalamus to increase the secretion of Growth Hormone Releasing Hormone (GHRH), the secretion of Growth Hormone (GH) in the pituitary will increase, where high levels of GH will stimulate the liver to secrete Insulin like Growth Factor-1 (IGF-1) which has the effect of inducing hypoglycemia and reducing glucogenesis so that blood glucose levels and insulin requirements decrease (Bunting et al., 2006). According to Rifai (2012), saponins play a role in increasing insulin secretion in the pancreatic Islet of Langerhans. According to Hernawan et al., (2004) stated that tannins that can be hydrolyzed are divided into gallotanins and ellagitannins. Gallotanin can increase glucose uptake while inhibiting adipogenesis and ellagitannin has properties like the insulin hormone (Insulin-like compound). Based on the results of research conducted by the treatment group, ethanol extract, n-hexane fraction, ethyl acetate and water had the effect of lowering blood glucose levels, this is in accordance with research conducted by Sai et al., (2021) which states that onco leaves contain flavonoids, alkaloids, saponins which are antioxidants and can reduce blood glucose levels.

The activity of reducing blood glucose levels in each treatment group can be related to antioxidant activity and the amount of pancreatic cell damage in the islets of Langerhans. Each treatment group, namely the positive control group, ethanol extract and ethanol extract fractions of onco leaves can regenerate and/or repair damaged pancreatic β cells so that they can produce insulin as evidenced by a decrease in blood glucose levels (Muthoharoh et al., 2022). Based on the results of research conducted, the best treatment group is the n-hexane fraction because the n-hexane fraction contains antioxidant compounds where alkaloids function to have an effect in inducing hypoglycemia and reducing glucogenesis so that blood glucose levels and insulin requirements decrease and saponins play a role in increasing secretion. insulin in the islets of Langerhans of the pancreas (Salehi et al., 2019). The mechanism of action is to help repair the β cells of the islets of Langerhans so that insulin production increases and protects pancreatic cells from free radicals (Prasetiyo et al., 2023). This is in accordance with research conducted by Sai et al (2021) that the onco leaf plant contains secondary metabolic compounds, namely flavonoids, saponins and alkaloids which It is an antioxidant and can lower blood glucose levels (Sai et al., 2021).

Each group treated with ethanol extract and the ethanol extract fractions of onco leaves had antihyperglycemic activity, although it was not comparable to the positive control (Elya et al., 2012). Although the drug glibenclamide does not play a direct role in regenerating pancreatic cells, this drug has the function of lowering blood glucose levels by stimulating insulin secretion (Rura et al., 2023). This is also in accordance with the decrease in blood glucose

levels in mice, where the treatment group experienced a decrease in blood glucose levels which was significantly different from the other groups (Riyaphan et al., 2021).

DM disease causes a condition where the body cannot control blood glucose levels so that insulin secretion by β cells in the islets of Langerhans pancreatic tissue becomes disturbed (Salehi et al., 2018). For the survival of cells in the body, glucose metabolism is needed. However, in a state of DM, the body's cells cannot metabolize glucose so the body becomes deficient in energy. This will respond to the body to look for alternative energy, namely from glycogenolysis and glycogenesis. Both products can produce free radicals which can cause damage to body cells including pancreatic β cells (Liu et al., 2016).

CONCLUSION

Based on the research results, it can be concluded thatEthanol extract and fractions from onco leaves (Spondias pinnata (Lf) Kurz) have antidiabetic activity with a working mechanism of inhibiting the alpha glucosidase enzyme. The n-hexane fraction from Spondias pinnata (Lf) Kurz) onco leaves has the activity of reducing blood glucose levels in male white rats (Rattus norvegicus) induced by streptozotocin. The n-hexane fraction from onco (Spondias pinnata (Lf) Kurz) leaves has the ability to improve the pancreatic histopathology of male white rats (Rattus norvegicus) induced by streptozotocin.

REFERENCES

Aguila-Muñoz, D. G., Jiménez-Montejo, F. E., López-López, V. E., Mendieta-Moctezuma, A., Rodríguez-Antolín, J., Cornejo-Garrido, J., & Cruz-López, M. C. (2023). Evaluation of α-Glucosidase Inhibition and Antihyperglycemic Activity of Extracts Obtained from Leaves and Flowers of Rumex crispus L. *Molecules*, *28*(15), 5760. https://doi.org/10.3390/molecules28155760

Alqahtani, A. S., Hidayathulla, S., Rehman, M. T., ElGamal, A. A., Al-Massarani, S., Razmovski-Naumovski, V., Alqahtani, M. S., El Dib, R. A., & AlAjmi, M. F. (2019). Alpha-Amylase and Alpha-Glucosidase Enzyme Inhibition and Antioxidant Potential of 3-Oxolupenal and Katononic Acid Isolated from Nuxia oppositifolia. *Biomolecules*, *10*(1), 61. https://doi.org/10.3390/biom10010061

Bunting, K., Wang, J. K, and Shannan, M.F. (2006). *Control of Interleukin-2-gene Transcription*: A Paradigm for Inducible. Tissue Speciic Gene Expression. Elsevier Academic Press. 74: 105-145

Daou, M., Elnaker, N. A., Ochsenkühn, M. A., Amin, S. A., Yousef, A. F., & Yousef, L. F. (2022). In vitro α-glucosidase inhibitory activity of Tamarix nilotica shoot extracts and fractions. *PLOS ONE*, *17*(3), e0264969. https://doi.org/10.1371/journal.pone.0264969

Dona, R., Fadhli, H., Furi, M., & Viryana, T. (2021). Uji Ekstrak Etanol Serta Fraksi Buah Kedabu (Sonneratia Ovata Backer) Sebagai Inhibitor Enzim A- Glukosidase.

Elya, B., Malik, A., SeptiMahanani, P. I., & Loranza, B. (2012). *Antidiabetic Activity Test by Inhibition of α-Glucosidaseand Phytochemical Screening from the Most Active Fraction of Buni (AntidesmabuniusL*. *4*(4).

Fitrilia, T., & M.Bintang. (2017). Inhibisi Enzim A-Glukosidase Menggunakan Ekstrak Daun Benalu Cengkeh (Denpropthoe pentandra (L.) Mic). *J Agroindustri Halal*, 41.

Hazra, B. B. (2008). Antioxidant and free radical scavenging activity of Spondias pinnata. *BMC Complementary and Alternative Medicine*, 8 .

Jain Preeti, Khondker Rufaka Hossain, Tamanna Rashid Mishue. (2014). Antioxidant and Antibacterial Activities of Spondias pinaata Kurz. Leaves. *European Journal of Medicinal Plants*, 4 920: 183-195.

Khalid, M., Alqarni, M. H., Alsayari, A., Foudah, A. I., Aljarba, T. M., Mukim, M., Alamri, M. A., Abullais, S. S., & Wahab, S. (2022). Anti-Diabetic Activity of Bioactive Compound Extracted from Spondias mangifera Fruit: In-Vitro and Molecular Docking Approaches. *Plants*, *11*(4), 562. https://doi.org/10.3390/plants11040562

Liu, S., Yu, Z., Zhu, H., Zhang, W., & Chen, Y. (2016). In vitro α-glucosidase inhibitory activity of isolated fractions from water extract of Qingzhuan dark tea. *BMC Complementary and Alternative Medicine*, *16*(1), 378. https://doi.org/10.1186/s12906-016-1361-0

Mohamed, E. A., Ahmad, M., Ang, L. F., Asmawi, Mohd. Z., & Yam, M. F. (2015). Evaluation of *α* -Glucosidase Inhibitory Effect of 50% Ethanolic Standardized Extract of *Orthosiphon stamineus* Benth in Normal and Streptozotocin-Induced Diabetic Rats. *Evidence-Based Complementary and Alternative Medicine*, *2015*, 1–6. https://doi.org/10.1155/2015/754931

Muthoharoh, B. L., Yuriah, S., Gustiani, R., Agustina, Y. R., Indrawati, I., & Mufdlilah, M. (2022). Efficacy of early initiation of breastfeeding (EIB) for preventing hypothermia in newborns. *Journal of Health Technology Assessment in Midwifery*, *5*(2), 82–95. https://doi.org/10.31101/jhtam.2211

Najihudin, A., Chaerunisaa, A., & Subarnas, A. (2017). Aktivitas Antioksidan Ekstrak Dan Fraksi Kulit Batang. *Indonesian Journal of Pharmaceutical Science and Technology*, 70-78.

Niaz, A., Adnan, A., Bashir, R., Mumtaz, M. W., Raza, S. A., Rashid, U., Tan, C. P., & Tan, T. B. (2021). The In Vitro α-Glucosidase Inhibition Activity of Various Solvent Fractions of Tamarix dioica and 1H-NMR Based Metabolite Identification and Molecular Docking Analysis. *Plants*, *10*(6), 1128. https://doi.org/10.3390/plants10061128

Nugrahaningsih, Ari Yuniastuti. (2014). Identifikasi Apoptosis Dengan Metode Tunel Pasca Pemberian Ekstrak Sambiloto Dan Pengaruhnya Terhadap Volume Tumor. *Jurnal Sains dan Teknologi.* 12: 139-146

Prasetiyo, A., Winarti, W., & Agustia, R. (2023). The inhibition of α-Glucosidase enzyme activity from Standardised Ethanol Extract of Abelmoschus manihot (L.) Medik Leaves. *JURNAL ILMU KEFARMASIAN INDONESIA*, *21*(2), 159. https://doi.org/10.35814/jifi.v21i2.1387

Riyaphan, J., Pham, D.-C., Leong, M. K., & Weng, C.-F. (2021). In Silico Approaches to Identify Polyphenol Compounds as α-Glucosidase and α-Amylase Inhibitors against Type-II Diabetes. *Biomolecules*, *11*(12), 1877. https://doi.org/10.3390/biom11121877

Rouzbehan, S., Moein, S., Homaei, A., & Moein, M. R. (2017). Kinetics of α-glucosidase inhibition by different fractions of three species of Labiatae extracts: A new diabetes treatment model. *Pharmaceutical Biology*, *55*(1), 1483–1488. https://doi.org/10.1080/13880209.2017.1306569

Rura, S. R., Djabir, Y. Y., Rahim, A., Ningsih, S., & Firdausi, N. (2023). Evaluation of the Anti-Diabetic Activity of Paliasa Leaf Ethanol Fraction (Kleinhovia hospita L) Through Inhibition of the Enzymes α-Glucosidase and α-Amylase. *Jurnal Ilmiah Kesehatan (JIKA)*, *5*(2), 342–351. https://doi.org/10.36590/jika.v5i2.509

Sai, K., Chhetri, S. B. B., Devkota, S. R., & Khatri, D. (2021). Evaluation of the Hypoglycemic Potential of Leaves Extract of Spondias pinnata (L.f.) Kurz. From Nepal. *The Scientific World Journal*, *2021*, 1–6. https://doi.org/10.1155/2021/3230351

Salehi, Ata, V. Anil Kumar, Sharopov, Ramírez-Alarcón, Ruiz-Ortega, Abdulmajid Ayatollahi, Tsouh Fokou, Kobarfard, Amiruddin Zakaria, Iriti, Taheri, Martorell, Sureda, Setzer, Durazzo, Lucarini, Santini, Capasso, … Sharifi-Rad. (2019). Antidiabetic Potential of Medicinal Plants and Their Active Components. *Biomolecules*, *9*(10), 551. https://doi.org/10.3390/biom9100551

Salehi, B., Kumar, N., Şener, B., Sharifi-Rad, M., Kılıç, M., Mahady, G., Vlaisavljevic, S., Iriti, M., Kobarfard, F., Setzer, W., Ayatollahi, S., Ata, A., & Sharifi-Rad, J. (2018). Medicinal Plants Used in the Treatment of Human Immunodeficiency Virus. *International Journal of Molecular Sciences*, *19*(5), 1459. https://doi.org/10.3390/ijms19051459

Yuriah, S. (2024). Hubungan Tingkat Pengetahuan Ibu Hamil Tentang Hiv/Aids Dengan Sikap Terhadap Provider Initiated Test And Counselling (PITC). *13*.

Yuriah, S., Juniarti, S., & Sepriani, P. (2023). Midwifery care for Mrs "Y" at BPM Soraya Palembang. *International Journal of Health Sciences*, *7*(S1), 2966–2984. https://doi.org/10.53730/ijhs.v7nS1.14631

Yuriah, S., & Kartini, F. (2022). Factors Affecting With The Prevalence Of Hypertension In Pregnancy: Scoping Review. *PLACENTUM: Jurnal Ilmiah Kesehatan Dan Aplikasinya*, *10*(1), 1. https://doi.org/10.20961/placentum.v10i1.54822

Yuriah, S., Kartini, F., & Isnaeni, Y. (2022). Experiences of women with preeclampsia. *International Journal of Health & Medical Sciences*, *5*(3), 201–210. https://doi.org/10.21744/ijhms.v5n3.1901