

Application of Africa Leaf Extracts (*Vernonia Amygdalina Delile*) as Diabetes Medicine and Identification of Activated Compounds

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Abstract - Diabetes mellitus (DM) is a disease associated with lifestyle, especially food consumption. One way to treat the pathogenesis of diabetes is by providing antioxidants which are proton compounds to reduce the negative effects of ROS, so that antioxidants can prevent interactions between radicals. African leaves contain flavonoid compounds, glycosides, saponins, tannins, and triterpenoids/steroids that function as primary antioxidants and scavenger to superoxide anions. The purpose of this study was to apply the African leaves extract (*Vernonia amygdalina* Delile) as a drug of diabetes mellitus and identify the active compound. The results showed that African leaves had an effect on blood glucose levels of diabetic rats and the concentration of African leaves extract which had an effect on the decrease of glucose level was 60% extract and began to show a decrease at the time of process 30 minutes to 150 minutes with glucose level 75 mg / dl . The content of African leaves extract (*Vernonia amygdalina* Delile) phytochemical results showed the presence of flavonoid, terpenoid, phenolic, alkaloid, and saponin compounds that function as antioxidants.

Keywords- African leaves, extraction, diabetes, phytochemical test

I. INTRODUCTION

Diabetes mellitus is a chronic disease caused by the inability of the pancreas to produce enough insulin or the body is unable to effectively use its insulin. Diabetes mellitus is a metabolic disorder or the way the body digests food into energy. According to Rational Presentation Information Media for Indonesian Health Workers (2001), glucose enters the cell via two ways: passive diffusion and active transport. In passive diffusion, the entry of glucose depends on the difference in glucose concentration between extracellular and in-cell media. In active transport, insulin acts as a facilitator on a particular tissue network. Insulin is the main anabolic hormone that increases energy reserves. In all cells, insulin increases the action of enzymes that convert glucose into a more stable form of energy reserves (glycogen). Hyperglycemia in diabetes mellitus is caused by a lack of insulin secretion by the Langerhans island beta

cells or the inability of insulin secretion to stimulate cellular blood sugar uptake.

Blood glucose levels into an indicator of the lack of food intake as a source of energy. The factor that determines the blood glucose level is the balance between the amount of glucose entering and the glucose secreted through the bloodstream. It is influenced by food, the speed of entry into muscle cells, fat tissue and other organs and the activity of glycogen synthesis of glucose by the liver.

Some of the mechanisms that contribute to the pathogenesis of diabetes mellitus due to hyperglycemia are glycation, lipid peroxidation, oxidation and DNA methylation resulting in DNA adducts and glucose autooxidation. The condition of diabetes also increases inflammatory compounds such as IL-6 (Interleukin 6) and TNF- α (Tumor Necrosis Factor α). ROS can induce NF- κ B

(Nuclear Factor kappa β) to express the cytokine compound gene [1]. ROS can be neutralized by the antioxidants present in the body so that biological damage by free radicals and radical compounds that can trigger the occurrence of degenerative diseases can be avoided [2].

One of plant that has the potential to be developed as a functional beverage is African leaves (*Vernonia amygdalina Delile*). African leaves are bush plants originating from the African continent and other parts of Africa, especially Nigeria, and tropical countries such as Indonesia. These plants can be found in the yard, along the rivers and lakes, on the edge of the forest, and in the meadows [3].

African leaves have upright plant stems, 1-3 m high, round, woody, brown; compound leaf, child opposite leaf, 15-25 cm long, 5-8 cm wide, shaped like the tip of a spear, jagged edges, pointed tip, rounded base, pinnate spine, dark green, taproot, dirty brown. African leaves plants contain many nutrients and chemical compounds, among others as follows: protein (19.2%), fiber (19.2%), carbohydrates (68.4%), fats (4.7%), ascorbic acid 166.5 mg/100 g), carotenoids (30 mg/100 g), calcium (0.97 g/100 g), iron (7.5 mg/100 g), phosphorus, potassium, sulfur, sodium, manganese, copper, zinc, magnesium and selenium. The chemical compounds contained in African leaves include saponins (vernionioside and steroidal saponins), sesquiterpenes (vernolide, vernoladol, vernolepine, vernodaline and vernomygin), flavonoids, coumarin, phenolic acids, lignans, xanthones, terpenes, peptides and luteolin. The aim of this research is to apply African leaves extract (*Vernonia amygdalina Delile*) as diabetes mellitus drug and identify active compound.

II. RESEARCH METHODS

A. Materials and Tools

The main ingredients used in this study were: African leaves (*Vernonia amygdalina Delile*), Alloxan (Sigma A7413-10G) to make normal mice become diabetic. The equipment used is mouse weight, glucometer (One Touch Ultra) for blood glucose measurements, syringes for injection of alloxan, Sonde for beverage, cage.

B. Preparation of Test Material

1000 gr of dried African leaves (*Vernonia amygdalina Delile*) boiled with 3 liters aquades for 10 minutes and filtered. The boiling result was concentrated with vacuum evaporator up to 500 ml. The obtained African leaves extract is packaged in polyethylene plastic and stored at 4 °C.

C. Testing the Influence Of African Leaves

After passing the adaptation period, 30 rats were made into diabetes by induction using alloxan monohydrate, induction was done by intraperitone injection, alloxan dose was used as much as 2.5 ml (Table 1). Tests were performed to see the effect of African leaves on blood glucose levels of diabetic rats before and after treatment.

Thirty of diabetic rats and one normal mouse were used in this study. Blood glucose measurement by glucometer using electrochemical method, that is based on potential measurement (electric power) caused by reaction from glucose with glucose reagent material on strip electrode. The test strip contains chemicals: glucose oxidase 29.1% w / w, 32.0% w / w and 32.9% w / w of non-reactive materials (38.9% w / w).

The working principle is the blood sample is absorbed into the end of the test strip based on the capillary reaction. When the blood fills the reaction chamber on the test strip, potassium fericyanide is decomposed and the sample glucose is oxidized by the glucose oxidase enzyme, causing a decrease in oxidation number (potassium hexanoanoferate (III) to potassium hexanoanoferate (II)). The application of constant voltage quantities of the meter oxidizes potassium hexanoanoferate (II) back in potassium hexanoanoferate (III), and gives electrons. The electrons generated to generate current are proportional to the glucose levels in the sample. After 60 seconds, the glucose concentration in the sample is displayed on the monitor screen.

D. Phytochemical Test

Test alkaloid compounds using the Meyer reagent. The meyer reagent was prepared by dissolving 0.2 grams of HgCl_2 with 6 ml of distilled water and as much as 0.5 g of KI dissolved in 1 ml of distilled water. Both solutions are mixed. The stages for the alkaloid test are the dissolved sample with 0.1 N hydrochloric acid and then macerated for 2 hours.

The maceration result is added 2-3 drops of meyer reagent. The presence of yellow color signifies positive alkaloids.

Test of Terpenoid Compounds, Steroids, Phenolics, and Flavonoids. The samples were shaken strongly with chloroform and added aquadest solution shaken and allowed to form two layers. The chloroform layer is dropped on the drop plate and allowed to dry, add a few drops of anhydrous acetic acid and concentrated sulfuric acid. The formation of red or orange signifies positive terpenoid compound and blue or green positive for steroids. A few drops of water

layer placed in a test tube are added iron (III) chloride solution, if green to purple indicates a phenolic positive. A few drops of water layer are placed in the test tube, added concentrated hydrochloric acid and magnesium powder, if a red color indicates a positive flavonoid compound.

Saponin test, a few of crude extract African leaves is extracted with diethyl ether. Then the soluble fraction in diethyl ether is separated. The residual residue that was not soluble in diethyl ether was added 5 ml of distilled water and then shaken, positive results were shown by stable foaming for 15 minutes.

III. RESULTS AND DISCUSSION

A total of 1 kg of African leaves taken and sieved and extracted using 3 liters of aquades and obtained an extract of 500 ml. The extracts were then diluted with concentrations of 12%, 24%, 36%, 48% and 60%. The process of making mice became diabetic by induced using Alloxan monohydrate. Induction is performed by intraperitone injection. African leaves extract was then given using sonde and measured glucose levels during processing time. The results of African leaves testing of diabetic mellitus rats can be seen in Table 1. Based on the data, can be made histogram effect of African leaves during the processing time (Fig 1). The results show that African leaves extract gives effect to diabetic mellitus rats and the longer the glucose level at animal tests provide significant results.

Table 1. Test Results of The Effect of African Leaves

Code Sample	Extract Concentration (%)	SZT Volume (ml)	Glucose level before treatment (mg/dl)	Glucose level after + SZT (5') (mg/dl)	Glucose level before treatment in time (mg/dl)				
					30'	60'	90'	120'	150'
A	12	2,5	119	124	121	168	171	168	162
B	24	2,5	117	127	135	154	160	158	157
C	36	2,5	120	125	135	149	176	168	150
D	48	2,5	121	123	136	139	143	108	94
E	60	2,5	122	124	138	127	116	89	75
Control	0	0	121						

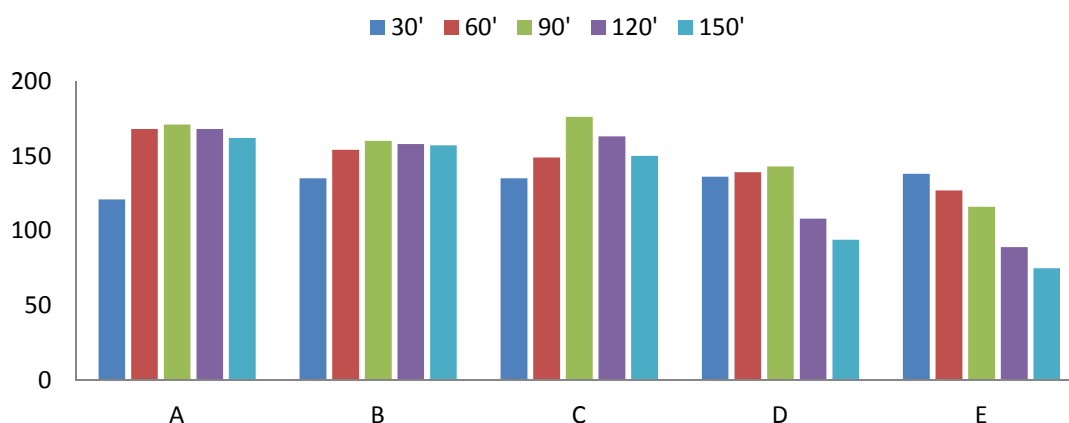


Figure 1. Histogram of Glucose Levels During Processing Time

Table 1 shows an increase in glucose levels before treatment and after the addition of SZT treatment as measured in the first 5 minutes. The data showed no

significant increase because it ranged from 2-5 mg / dl. This is because the effect of adding SZT has not worked in the span of 5 minutes.

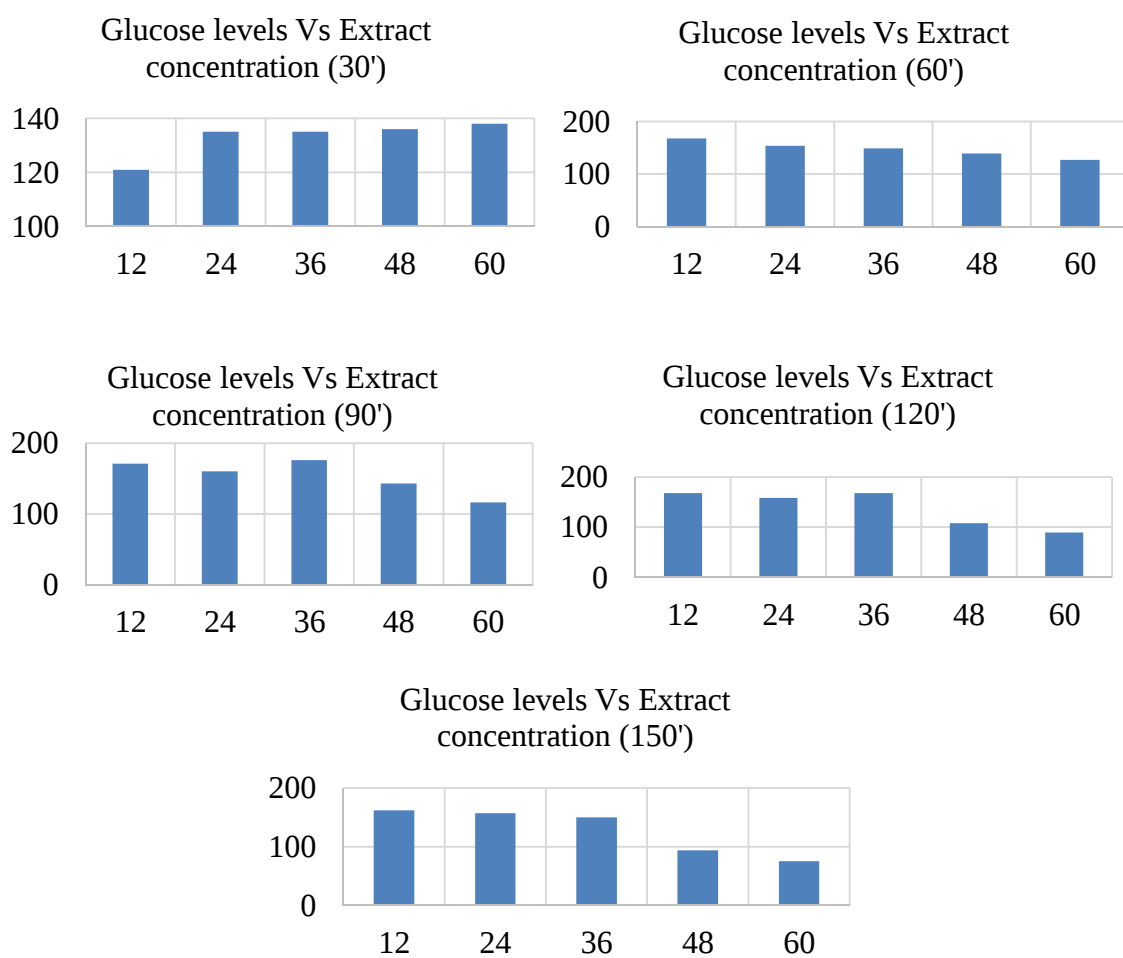
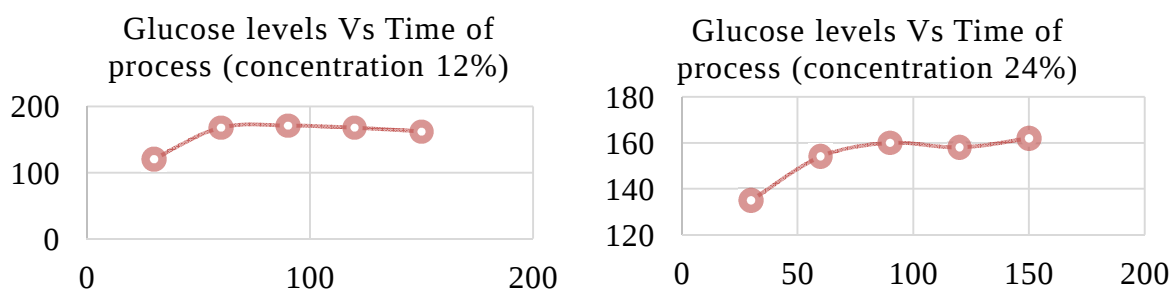


Figure 2. Histogram Glucose Levels vs Concentration of Extracts During Processing Time

After the treatment of African leaves extract with several concentrations and measured at 30 minutes showed elevated glucose levels, because the effects of SZT administration started to work and the effect of African leaves did not yet exist. The effect of extract concentration during processing

time 30 minutes showed the lowest concentration was 12%, for 60 minutes processing time the lowest concentration was 60% and up to 150 minutes process time obtained the best concentration was 60% (Fig 2).



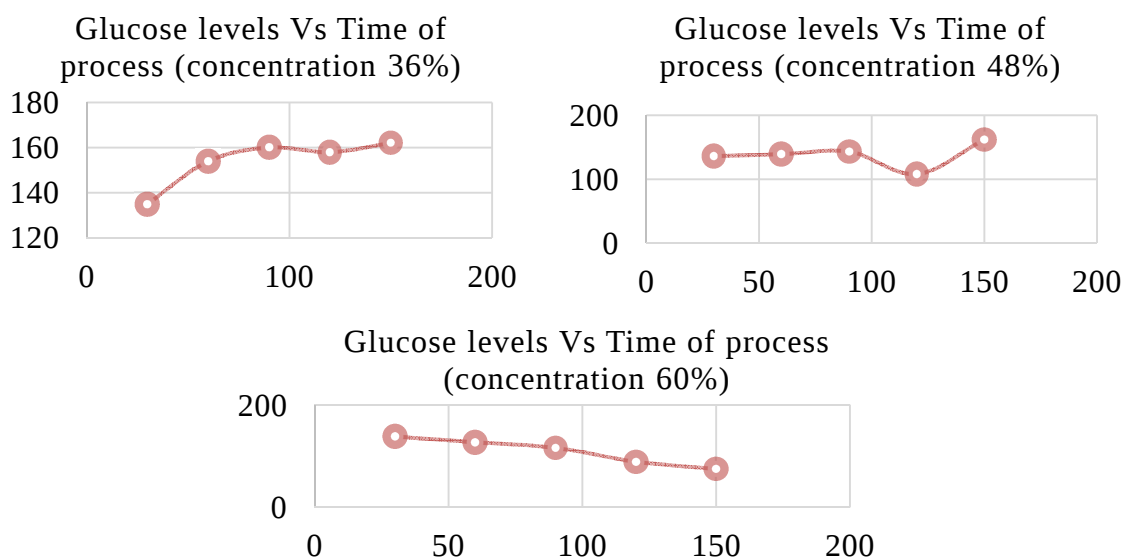


Figure 3. Graph of Glucose Level Vs. Time of Process

In Figure 3, it is seen that giving of African leaves extract at 12% concentration has less effect on glucose level and decreased glucose level is very slow. The same is true for concentrations of 24%, 36% and 48%. The best concentration that gives effect to the decrease of glucose level is 60% and starts to see decrease at process time 30 minutes to 150 minutes with glucose level 75 mg/dl. The content of African leaves extract (*Vernonia amygdalina* Delile) result of phytochemical test (Table 2) showed the presence of flavonoid, terpenoid, phenolic, alkaloid, and saponin compounds. Flavonoid compounds serve as primary antioxidants and scavenger to superoxide anions [4]. Flavonoid acts as a hydrogen donor, has the ability to stabilize and delocalize unpaired electrons in free radicals, and is able to chelate metal ions (Fenton's termination termination)[5]Table 2. Results of Phytochemical Test of African Leaves (*Vernonia amygdalina* Delile)

No.	Test Parameters	Color	Result
1	Flavonoid	Yellow	+
2	Steroid	Red	-
3	Terpenoid	Red	+
4	Phenolik	Blue	+
5	Alkoloid	Yellow	+
6	Saponin	Foam	+

IV. CONCLUSION

African leaves give effect to blood glucose level of diabetic rats and concentration of African leaves extract

which gives effect to decrease glucose level is 60% extract and start to see decrease at process time 30 minutes until 150 minutes with glucose level 75 mg/dl. The content of African leaves extract (*Vernonia amygdalina* Delile) phytochemical results showed the presence of flavonoid, terpenoid, phenolic, alkaloid, and saponin compounds that function as antioxidants.

ACKNOWLEDGMENTS

To Rector of Pattimura University, Research and Devotion Institute of Pattimura University, Ministry of Research, Technology and Higher Education who has funded the research in accordance with letter of the Rector Decision No: 605 / UN13 / SK / 2017.

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