

Bioactivity of Papua Red Fruit Extract (*Pandanus conoideus* L.) Against Superoxide dismutase, Malondialdehyde and Blood Glucose of Rat (*Rattus norvegicus* L.) HyperglycemiaMokosuli Yermia Samuel^{1,3*}, Natalina Migau^{1*}, and Masje Wurarah²,¹Master of Biology Study Program, Faculty of Mathematics Natural and Earth Sciences, Universitas Negeri Manado, Tondano 95619, Indonesia.²Biology Education Study Program, Faculty of Mathematics Natural and Earth Sciences, Universitas Negeri Manado, Tondano 95619, Indonesia.³Bioactivity and Molecular Biology Laboratory, Faculty of Mathematics Natural and Earth Sciences, Universitas Negeri Manado, Tondano 95619, Indonesia.*Corresponding author email: yermiamokosuli@unima.ac.id**Received** July 09, 2023; **Accepted** February 04, 2024; **Available online** March 20, 2024

ABSTRACT. Red fruit (*Pandanus conodus* L.) is a plant native to Papua, ethnomedical used as a traditional medicine with a wide spectrum of pharmacological effects. However, research on the bioactivity of red fruit is still very poorly reported. The aim of this study was to characterize the bioactive content, antioxidant bioactivity and the effect of lowering blood glucose in hyperglycemic rats. Red fruit has extracted used the maceration method with 95% ethanol. Analysis of active compound content using Gas Chromatography Mass Spectrophotometry (GC MS). Analysis of Malondialdehyde (MDA), Superoxide dismutase (SOD) and blood glucose levels was carried out using the in vivo method using white rats. The research results showed that red fruit ethanol extract contained 173 compounds from six main groups of compounds. Red fruit extract has effective in reducing MDA levels in hyperglycemic mice. K4 and K5 treatments were the best treatments for reducing MDA. Red fruit extract affects the duration of induction of increased SOD in hyperglycemic rats. Treatments K4 and K5 were the best treatments for SOD induction. Red fruit extract affects reducing blood glucose in hyperglycemic mice. Red fruit extract has antioxidant activity and lowers blood glucose. Red fruit extract has the potential to be developed as a source of bioactive antioxidants and antidiabetics.

Keywords: Glucose, hyperglycemia, malondialdehyde, superoxide dismutase**INTRODUCTION**

A severe metabolic disorder known as diabetes mellitus (DM) is caused by the body not being able to use insulin or the pancreas not producing any insulin at all. The prevalence of DM is rising to epidemic levels. According to the International Diabetes Federation (IDF), 463 million individuals worldwide were diagnosed with diabetes in 2019. This number is expected to rise to 700 million by 2045. Type 2 diabetes is estimated to account for the majority of cases (Saeedi et al., 2019). As DM is linked to a higher risk of micro- and macrovascular complications (such as retinopathy, neuropathy, and nephropathy), as well as cardiovascular disease (involving the heart, cerebrovascular, and peripheral arteries), the projected increase in incidence will lead to higher medical care costs (Bommer et al., 2018), a lower quality of life, and a higher mortality rate (Makrilakis et al., 2018).

Hyperglycemia is the technical term for high blood glucose (blood sugar). High blood glucose occurs when the body has too little insulin or when the body

cannot use insulin properly (Taylor et al., 2021; Bourebaba et al., 2021). Hyperglycemia is one of the hallmarks of DM disease. Hyperglycemia occurs due to an increase in blood glucose levels beyond normal limits. Hyperglycemia is a state of increased fasting blood glucose levels exceeding 126 mg/dL or when blood glucose levels exceed 200 mg/dL (Kasavadev et al., 2021).

Continuous hyperglycemia can cause problems in other organs of the body. Drugs are the only alternative to reduce blood sugar for people allergic to insulin injections. However, side effects are possible with chemical synthesized medications. High doses might cause hypoglycemia, liver issues, and diarrhea. Therefore, we need an alternative treatment that is safe and can be consumed for a long time. Hyperglycemia can be triggered by oxidative stress. High blood glucose levels can auto-oxidize, which produces free radicals (Mahmud et al., 2023).

Oxidative stress is a state of imbalance between free radicals and antioxidants. The production of free radicals in diabetes mellitus, which occurs due to

glucose auto-oxidation, exceeds the ability of intracellular antioxidants to neutralize them, causing cell damage. Oxidative stress can trigger lipid peroxidation (Chaudhary et al., 2023). Lipid peroxidation alters cells, developing toxic metabolites, increasing membrane permeability, decreasing calcium transport in the sarcoplasmic reticulum, and decreasing the activity of mitochondria and enzymes (Berawi and Agyerianti, 2017). As the cell structure changes, its function is also disturbed, which triggers the development of degenerative diseases. Antioxidants are needed to suppress the increased production of free radicals (Martemucci et al., 2022).

Antioxidants can bind free radicals to reduce the risk of type 2 DM and help reduce insulin resistance. Antioxidants contained in the body must be present in sufficient quantities to fight free radicals. Both enzyme- and non-enzyme-based antioxidants make up the endogenous antioxidant defense mechanism. Antioxidants that are produced by enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GSR) (Wilcox et al., 2004; Papachristoforou, et al., 2020). Antioxidants classified as metabolic and nutritional antioxidants are nonenzymatic (Finley et al., 2011).

Lipoic acid, glutathione (GSH), L-arginine, coenzyme-Q10, melatonin, uric acid, bilirubin, metal chelating proteins, and transferrin are a few examples of metabolic antioxidants that the body produces throughout metabolism, including endogenous antioxidants (Halliwell, 2023). Contrarily, exogenous antioxidants are substances like vitamin E, vitamin C, carotenoids, lycopene, trace metals (selenium, manganese, zinc), flavonoids, omega-3 fatty acids, and omega-6 that the body cannot produce on its own and must be obtained through food or supplements. Exogenous antioxidants obtained from food are needed to counteract the effects of free radicals in the body (Martemucci et al., 2022).

One endemic plant that has the potential to be utilized for its antioxidant compounds is the Papuan red fruit. The spread of red fruit is most dominant around the mountains of Jayajaya, Jayapura, Manokwari, Nabire, Timika and Ayamaru-Sorong. Traditionally, the people of Papua consume red fruit from those who live in coastal and mountainous areas; apart from being a source of food from generation to generation, it is also used as a natural food colouring and craft material (Zebua and Walujo, 2016). Traditional communities living in the Jayawijaya mountainous region, such as Wamena and Tolikara, who make red fruit an ingredient in their daily diet, have a more muscular and strong physique than people from other regions in Papua. Even so, there are still very few scientific reports on the use of red fruit for diabetes mellitus.

Antioxidant synthetic and natural compounds can control blood glucose and prevent further diabetes

complications (Erlidawati et al., 2018). Cinnamic acid, coumarin, diterpenes, flavonoids, polypropanoids, tannins, and triterpenes are some bioactive compounds with antioxidant properties. Secondary metabolite compounds that have antioxidant activity can be found in almost all parts of red fruit plants. Beta-carotene (700 ppm), tocopherol (500 ppm), total carotenoids (12,000 ppm), total tocopherols (11,000 ppm), and fatty acids like oleic acid (58%), linoleic acid (8.8%), linolenic acid (7.8%), and decanoic acid (2.0%) are just a few of the active substances found in red fruit at relatively high levels (Fadli, 2021).

Red fruit ethanol extract is an antioxidant with a capacity of $1,392 \times 10^{-3}$ g ATE/g extract (Wabula et al., 2019). Red fruit ethanol extract has activity in reducing LDL 0.50 g/200 BW of rats, which is 50.40 mg/dL (Fadli, 2021). Red fruit extract significantly reduces total cholesterol levels in white rats (*Rattus norvegicus* L.) with an atherogenic diet (Adetasia, 2021). Even so, there are still very few reports of the antioxidant activity of red fruit extract under certain conditions. A study has been conducted to determine the activity of red fruit extract in hyperglycemic rats on antioxidant activity and blood glucose content.

EXPERIMENTAL SECTION

Sample

Papuan red fruit was obtained from Nabire Papua in September 2022. Taxonomic analysis of red fruit was carried out in the Biology Laboratory of the Faculty of Mathematics of Natural and Earth Sciences, Manado State University, with a species identification letter number: 275/UN41.1.1./LB/2022.

Red Fruit Extract.

2.5 kg of red fruit was prepared, finely chopped, and blended. The simplicia was then oven-dried at 40-45 °C until the dried fruit was obtained. Extraction was carried out by the maceration method. As much as 500 g of red fruit simplicia was macerated with 3 L of 95% ethanol. The mixture was left for 2 x 24 hours in a shaker incubator at 45 rpm. The filtrate obtained was treated as a solvent with a Heidolp Rotary Evaporator at 55 rpm, 45 °C.

Analysis of Compound Content with GC MS.

Concentrated red fruit volatile extract was injected into the GC-MS. Separation of volatile components in the GC-MS capillary column was carried out by injecting one pL of sample into the GC MS instrument Shimadzu type QP 5000, which was operated using a splitless injection technique with a sampling time of 0.5 minutes in a DB5 capillary column with a length of 30 meters, an inner diameter of 0.25 mm and a film thickness of 0.25 p. The carrier gas is helium at a pressure of 40.40 kPa. The injector temperature is 230 °C, the interface temperature is 240 °C, and the oven temperature program is started at 50 °C, held for 5 minutes to 225 °C, held for 10 minutes. The

temperature gradient used was 3 °C/min. The detector used was MS (Mass-Spectrometer) with an ionization energy of 170 eV, a mass range of 33-400 in intervals of 0.5 seconds, a resolution of 1000. A mixture of one series of alkanes (C_n-C_n) was injected into the GC-MS under the same conditions as above to calculate the Linear Retention Indices (LRI) of the red fruit volatile components. Identification was carried out by comparing the mass spectra of the components in the sample with the mass spectra found in the NIST library with the help of Class 5000 software (Shimadzu). Confirmation of component identification was carried out by comparing LRI components with authentic component LRIs found in the literature (Gdwdader et al., 1994).

In Vivo Test

Red Fruit Extract Treatment and Blood Glucose Analysis

Fifteen white male rats (*Rattus norvegicus* L.) were divided into five cages and acclimated to laboratory animals. Intravenous alloxan induction in test animals to make the test animals experience hyperglycemia. Convert dosage in volume for glibenclamide and red fruit extract used. The treatment test phase consisted of acclimation of the test animals for seven days and intravenous induction of alloxan in the test animals at a dose of 14 mg/0.02 mL, which was carried out once and waited for three days (test animals were declared diabetic if their blood glucose levels were more than 200 mg/dL), and induction of red fruit extract in test animals with a predetermined dose series (Table 1); extract induction was carried out every day for 21 days by means of gastric sondage. On the 8th, 11th and 32nd day of the research treatment test stage, each test animal fasted for 10 to 12 hours for further blood sampling to be carried out via the tail vein. Analysis of glucose content was carried out at the Trinita University Laboratory using the GOD-PAP (enzymatic photometric test) method. Analysis of the fasting blood glucose of the test animals was carried out immediately after blood sampling and a maximum of 2 hours after blood sampling.

Measurement of SOD Levels.

Superoxide dismutase (SOD) levels were measured using Ransod Superoxide Dismutase Manual Rx Monza reagent. SOD levels were measured using a

UV-Vis spectrophotometer with a maximum wavelength of 505 nm and a temperature of 37°C. The plasma of the test animals was reacted with reagent R1a and reagent R1b, and then the absorbance was observed at 30 seconds and 180 seconds. Each sample has an absorbance value from 30 seconds after measurement (A1) and 3 minutes after (A2). Calculations are performed using the formula = A/min from the standard or sample. The sample activity value was converted to a percentage of the sample solvent value (S1) and subtracted from 100% to give the inhibition percentage value.

$$\% \text{ inhibition} = 100 - \frac{\text{A sample / min} \times 100}{\text{AS1 / min}}$$

Measurement of Malondialdehyde Levels.

Measurement of MDA levels using the UV Vis spectrophotometry method. Rats were acclimated for two weeks by ad libitum administration of commercial rations and drinking water. The rats were fasted for 8 hours, and the initial blood glucose levels were measured. Fifteen rats were induced using alloxan at a dose of 110 mg/KgBW intraperitoneally, and then after five days, the initial Malondialdehyde (MDA) levels were measured. Mice were divided into three groups of 5 rats each. The treatment given was as follows in Table 1.

After 14 days of treatment, the rats were injected with ketamine at 50 mg/kg BW intraperitoneally. Then the capillary tube is etched on the medial canthus of the eye under the eyeball towards the optic foramen. The capillary tube is rotated until it injures the plexus, and the blood is collected in a vial that has been given 10% EDTA as much as 1 ml. Blood was collected in vials containing 10% EDTA solution. Blood was put into a centrifuge tube, and then the protein was precipitated by adding 2.5 ml of 10% trichloroacetic acid. Then it was centrifuged at 1000 rpm for 10 minutes; the supernatant was separated, and the protein deposits were taken. The protein precipitate was added with 2.5 ml of 10% acetic acid and 3 ml of thiobarbituric acid. The mixture was heated in a water bath for 30 minutes and then cooled rapidly in an ice bath to stop the reaction. It was then extracted by adding 4 ml of n-butanol and centrifuged at 3000 rpm for 10 minutes. The results of the centrifuge were measured for absorption on spectrophotometry with a wavelength of 532 nm.

Table 1. Treatment in test rats

Treatment	Code	Description
Control (ddH ₂ O)	K1	ddH ₂ O
Alloxan	K2	Alloxan 110 mg/Kg BW
Glibenclamide	K3	Control positive
Treatment I	K4	200 mg/Kg BW
Treatment II	K5	400mg/Kg BW

RESULTS AND DISCUSSION

Red Fruit Extract

The red fruit is obtained directly from Nabire Regency, West Papua Province. Ripe red fruit was used in this study. The fruit hump is composed of many small stone fruits which are often called drupa. The color of the red fruit drupa used in this study was red. Fresh red fruit is cut into cubes and then dried at room temperature. Simplicia is in powder form, red in color, and has a distinctive aroma (**Figure 1**). The filtrate obtained after maceration for 2 x 24 hours is blood colored and has a distinctive red fruit aroma. Evaporation of the solvent with a rotary evaporator produces a blackish red crude extract (**Figure 2**):

Compound Content

Red fruit crude extract was successfully analyzed for compounds using GC MS. GC MS is widely used to analyze compounds in crude extracts. The crude extract is a mixture of various compounds. GC MS can read compounds with the lowest concentration so that secondary metabolites in an extract can be identified with the output in the form of chromatograms and mass spectra. The results of the GC MS analysis

obtained six main peaks. Six groups of compounds were obtained in the ethanol extract of red fruit. The most significant percentage of area is at the third peak, while the smallest is at the fifth peak (**Figure 3**).

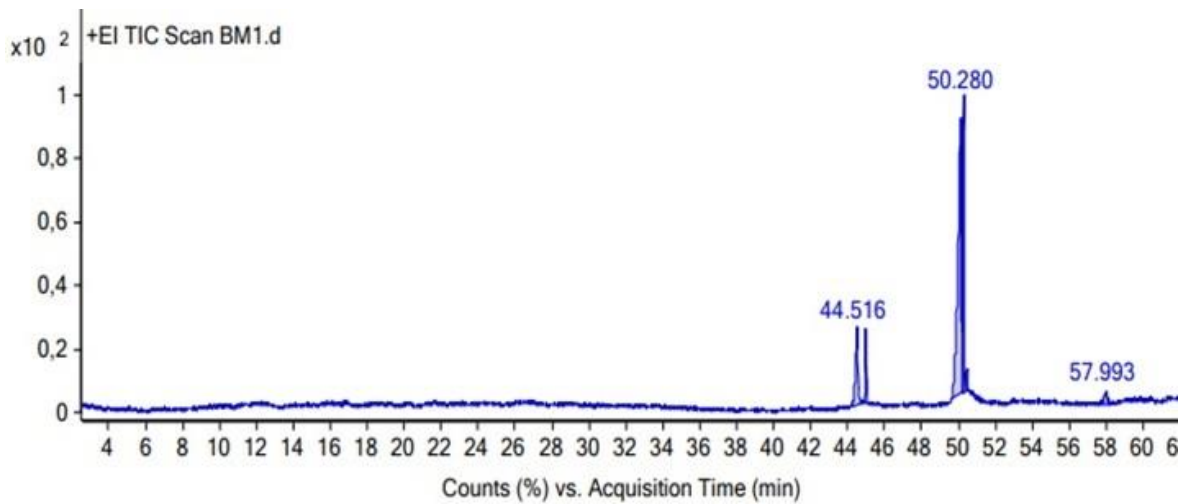
The MS spectrum output showed several groups of compounds, namely n-Hexadecenoic acid; Hexadecenoic acid, ethyl ester; oleic acid; 9-Octadecenoic acid, ethyl ester; 1,14-Tetradecanediol; 1,5-Heptadiene, 3,3-dimethyl. The spectrum of the library and the number of peaks in each group are shown in Table 1. The spectrum of the library shows the framing of compounds in one group. Based on the number of peaks produced, the compound in the n-Hexadecenoic acid group has 21 compounds. The hexadecenoic acid group, ethyl ester, has 30 compounds. The Oleic Acid group has 46 compounds. The 9-Octadecenoic acid group has 48 compounds. The 1,14-Tetradecanediol group has 24 compounds, and the 1,5-Heptadiene, the 3,3-dimethyl group, has four compounds. So that the total compounds contained in the ethanol extract of Papua red fruit based on the results of GC analysis are 173 compounds.



Figure 1. Preparation of Red Fruit Simplisia from Nabire Papua (a). Fresh red fruit (b). Red fruits are cut into rectangles and dried (c). Preparation of simplisia of red fruits in the form of powder.



Figure 2. Extraction of red fruits by maceration method. (a). Maceration 2 x 24 hours (b). Filtrate filtration (c). Crude extract after evaporation of solvent with a rotary evaporator.



Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	44208	44516	44772	193232	1801719	2064
2	44895	44977	45059	187547	713987	818
3	49624	50116	50198	692481	8728186	10000
4	50198	50280	50383	749917	3399548	3895
5	50383	50444	50546	53827	245047	281
6	57665	57993	58157	33767	407477	467

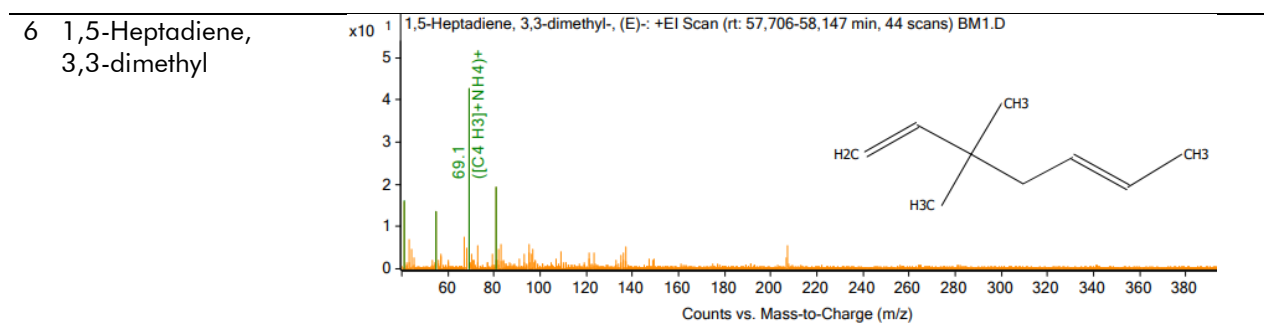
Figure 3. GC MS output red fruit ethanol extract

Table 2. Library Spectrum and Peak Number of each class of compounds

No.	Compound Classes	Spektrum Library	Number of Peaks
1	<i>n</i> -Hexadecanoic acid		21
2	Hexadecanoic acid, ethyl ester		30
3	Oleic Acid		46
4	9-Octadecenoic acid		48
5	1,14-Tetradecanediol		24
6	1,5-Heptadiene, 3,3-dimethyl		4

Table 3. Characteristics of GC MS Compound Groups

No	Compound Name	Characteristic
1	<i>n</i> -Hexadecanoic acid	<p>x10² <i>n</i>-Hexadecanoic acid: +EI Scan (rt: 44,321-44,608 min, 29 scans) BM1.D</p>
2	Hexadecanoic acid, ethyl ester	<p>x10² Hexadecanoic acid, ethyl ester: +EI Scan (rt: 44,926-45,029 min, 11 scans) BM1.D</p>
3	Oleic Acid	<p>x10² Oleic Acid: +EI Scan (rt: 49,767-50,198 min, 43 scans) BM1.D</p>
4	9-Octadecenoic acid	<p>x10² 9-Octadecenoic acid, ethyl ester: +EI Scan (rt: 50,198-50,321 min, 13 scans) BM1.D</p>
5	1,14-Tetradecanediol	<p>x10¹ 1,14-Tetradecanediol: +EI Scan (rt: 50,383-50,516 min, 14 scans) BM1.D</p>



The characteristics of the six groups of compounds were obtained, namely compounds one to five were fatty acid derivatives, while the sixth compound did not include fatty acids. Compounds of the Hexadecenoic acid group have 16 carbon atoms without double bonds. Hexadecenoic acid, ethyl ester has the same number of carbon atoms as Hexadecenoic with the addition of ethyl ester. Oleic Acid has 18 carbon atoms. 9-Octadecenoic acid has 20 carbon atoms without double bonds. 1,14-Tetradecanediol has 14 carbon atoms. 1,5-Heptadiene, 3,3-dimethyl has nine carbon atoms (**Table 2**).

GC-MS is a method of separating organic compounds that uses two methods of compound analysis: gas chromatography (GC) to analyze the number of compounds quantitatively and mass spectrometry (MS) to analyze the molecular structure of the analyte compound. Papuan red fruit extraction was successfully carried out using 95% ethanol. Ethanol is the most widely used solvent for plant extraction to test bioactivity. Extract weight and high yield percentage showed that extraction with 95% ethanol effectively extracted secondary metabolites in red fruit. GC MS analysis aims to identify the aromatic compounds in the red fruit extract. The results of the GC MS analysis identified 173 compounds. This shows that the compound content in the red fruit extract is very high. The compounds produced are derivatives of six main compound groups, all lipid derivatives.

MDA Content of Rat Blood

Malondialdehyde is a product formed from lipid peroxidation in cell membranes, namely the reaction of free radicals (hydroxyl radicals) with polyunsaturated fatty acids (PUFA). The average initial MDA level was in the range of 159.43 mg/dL (K1) to 188.89 mg/dL (K3). The highest mean final MDA was 194.57 mg/dL (K2), while the lowest was 111.24 mg/dL (K5). The highest average percentage difference in the decrease in blood MDA levels of rats was 32.52% (K5), while the lowest was 7.46% (K1). Compared to the positive control (K3), the average reduction in MDA of rats given red fruit extract intake was higher (**Figure 4**).

The analysis of variance showed that the red fruit extract treatment significantly affected MDA levels in rat blood ($p < 0.05$). Duncan's multiple range test (DMRT) follow-up tests showed that the K1 and K2 treatments were not significantly different. The K2

treatment was not significantly different from the K4 treatment. The K4 treatment was not significantly different from the K4 treatment. K3 Treatments K1, K2, K3 and K4 were significantly different from those of K5.

Analysis of the antioxidant potential of extracts in vivo in white rats showed that K4 and K5 extracts had the highest MDA reduction effect compared to other treatments. This indicates that the dose of red fruit extract given to white rats (k4 and K5) is the best extract in reducing MDA levels. Lipid peroxidation can be determined by measuring MDA, which is the product formed from the reaction of ROS with fatty acids from the cell membrane (Zengin, 2021; Zaloga, 2021). Lipid peroxidation will cause the breakdown of fatty acid chains into various toxic compounds and cause damage to cell membranes (Mas-Bargues et al., 2021). The K5 treatment showed the highest percentage difference in MDA reduction and was still better than the positive control. This means that the compound content in the red fruit extract can inhibit the lipid peroxidation process in the rats. The analysis of variance also showed that the extract treatment had a significant effect on the MDA levels of the rats ($p < 0.05$). The DMRT follow-up test also showed differences between the extract and control treatments. Superoxide dismutase (SOD is one of the intracellular antioxidant enzymes. K3 and K5 treated red fruit extracts showed an increase in the percentage of SOD levels.

SOD Content of Rat Blood

Superoxide dismutase (SOD) is an antioxidant enzyme that is a physiological defense strategy in animals and plants against free radicals and reactive oxygen species (ROS) resulting from biotic and abiotic stresses. The average initial SOD levels in rat blood for treatment K1 (83.33%), K2 (37.78%), K3 (35.37%), K4 (38.01%) and K5 (39.01%). The highest percentage reduction in SOD content based on the difference between initial SOD and SOD after intake of red fruit extract in rats was found in the K2 treatment, namely 2.41%. Whereas in control rats or without red fruit intake, there was an average decrease in SOD of 3.82%. Unlike the positive control (K3) there was an increase in blood SOD content of 74.26%. The increase also occurred in the red fruit extract treatment, namely on K4 and K5; there was an

increase in % blood SOD, respectively 96.82% and 103.92% (Figure 5). Thus, the intake of red fruit extract induces an increase in the percentage of blood SOD in rats. The results showed that treatment with red fruit extract increased the SOD content in rat blood compared to the initial conditions before the administration of red fruit extract.

The analysis of variance showed that red fruit extract treatment on rats affected white rat blood SOD levels ($p < 0.05$). The DMRT follow-up test showed that K2 treatment was not significantly different from K1. However, it was significantly different from other treatments. The induction of secondary metabolites in red fruit caused an increase in SOD levels in rats. The increase in SOD indicated an increase in rats' natural antioxidant system due to the intake of red fruit extract. Analysis of variance showed a significant effect of giving red fruit extract on SOD levels in rats ($p > 0.05$). The DMRT follow-up test also showed differences in treatment, where K4 and K5 treatments showed the best SOD induction compared to other treatments. The K5 extract showed the best blood glucose reduction parameter. Compared to the control glibenclamide, the percentage decrease in blood glucose in red fruit (K5) treated rats was still better. Analysis of variance also showed an effect of red fruit extract on differences in rat blood glucose levels. The DMRT test also showed differences in treatment, where the K5 treatment was the best in response to decreasing blood glucose levels in rats.

Blood Glucose Content

Blood glucose is the sugar found in the blood which comes from carbohydrates in food and is stored as glycogen in the liver and skeletal muscles. The lowest average blood glucose level after induction of

hyperglycemia was 269 mg/dL (K1), and the highest was 286.37 mg/dL (K3). After the administration of red fruit extract, the lowest average blood glucose was 120.40 (K5), and the highest was 224.67 (K2). The highest average percentage decrease in blood glucose in rats that were given red fruit extract was 57.51% (K5). Compared to the positive control, namely the administration of the antidiabetic drug glibenclamide, the percentage decrease was 45.72% (K3). Thus, intake of red fruit extract provides a better blood glucose-lowering effect (Figure 6).

Based on the analysis of variance, a significant value of 0,000 was obtained. Thus, the red fruit extract treatment significantly affected the blood glucose levels of white rats ($p < 0.05$). The results of the DMRT follow-up test showed that the K1 and K2 treatments were not significantly different; the K3 and K4 treatments were not significantly different. The K5 treatment was not significantly different from the K4 treatment but significantly different from all other treatments.

Based on the GC MS analysis, 172 compounds were obtained in the red fruit extract. The main compound group is n-Hexadecenoic acid; Hexadecenoic acid, ethyl ester; oleic acid; 9-Octadecenoic acid, ethyl ester; 1,14-Tetradecanediol; 1,5-Heptadiene, 3,3-dimethyl. The n-hexadecenoic acid compound isolated from *Bergenia ciliata* flowers exhibits anticancer, anti-inflammatory, anticancer, larvicidal and antidiabetic activities. Fatty acids from extra virgin olive oil can reduce blood glucose levels in rats induced by hyperglycemia (Astuti, 2018). Oleic acid from peanuts and pure olive oil can induce a decrease in blood glucose levels and suppress metabolic syndrome (Zhao et al., 2019).

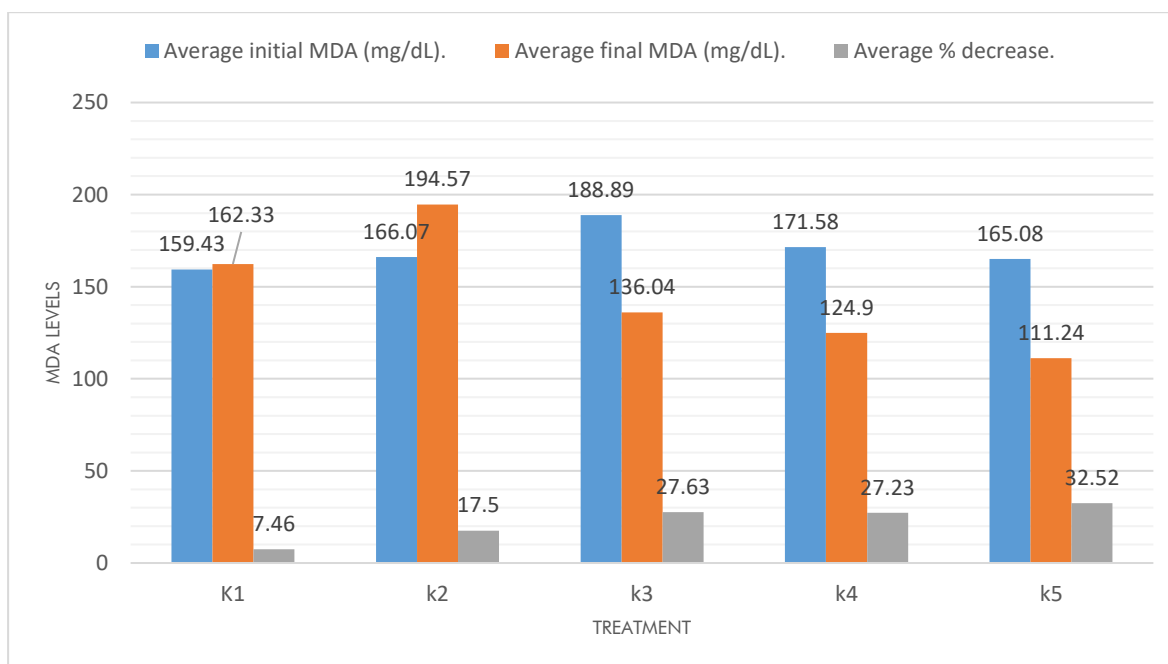


Figure 4. Percentage difference in MDA level reduction

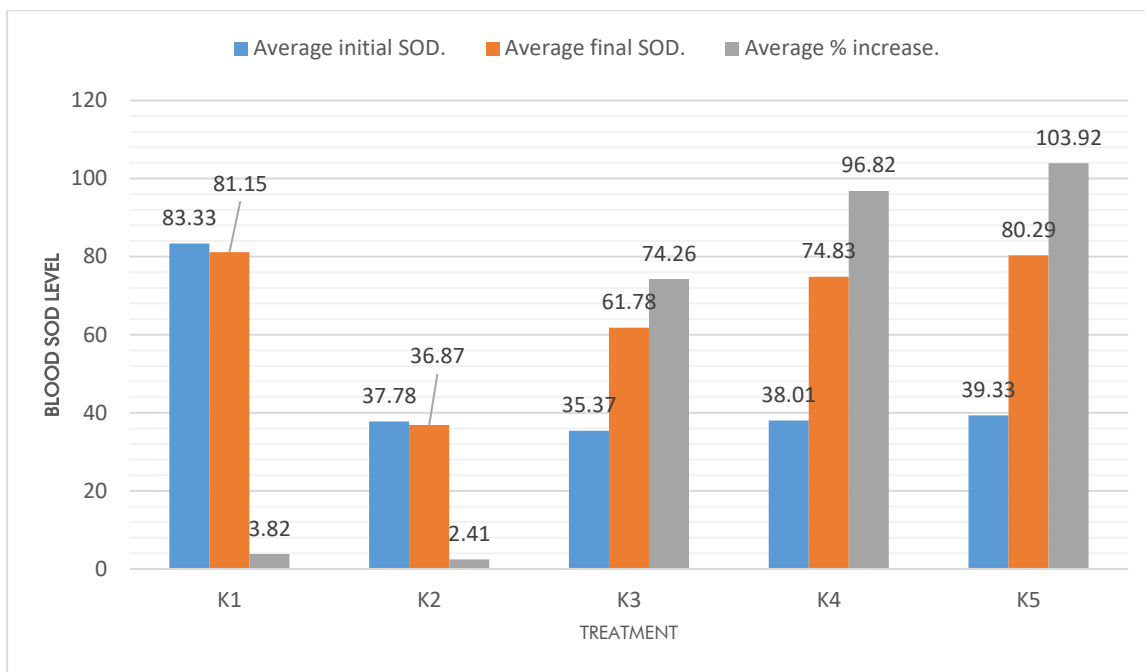


Figure 5. Comparison of the increase and decrease in rat blood SOD after treatment with red fruit extract intake

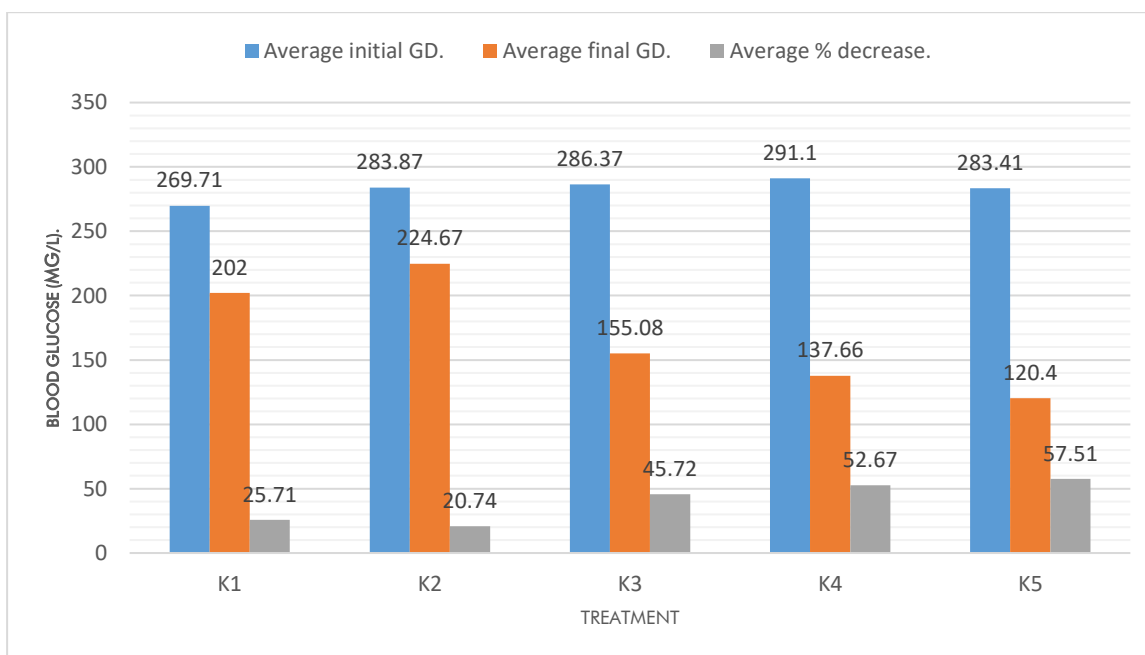


Figure 6. Comparison of the average percentage of blood glucose reduction per treatment

Bioactive 1,14-Tetradecanediol from *Polygonum glabrum* (Wild) shows antibacterial activity (Ezhilan, & Neelamegam, 2011); Anand and Gokulakrishnan, 2012). Fatty acid compounds from red fruit have antioxidant activity that can prevent the oxidation of unsaturated fatty acids. Antioxidants dissolve with triglycerides. During the separation process, antioxidants protect the triglycerides in the red fruit extract from oxidation reactions. So that at the end of the elution, triglycerides are obtained without destroying the compound (Gunawan et al., 2021). Non-polar red fruit extract based on the results of GC

MS GC-MS analysis obtained methyl palmitate, methyl oleate, methyl stearate, ethyl palmitate, ethyl oleate, aldehyde and cyclopentane aliphatic chains with carboxylic groups.

CONCLUSIONS

This study concluded that the red fruit ethanol extract contained 173 derivative compounds of 6 main group compounds. Red fruit extract had a significant effect on reducing MDA levels in hyperglycemic rats. The K45 treatment was the best treatment for reducing MDA. Red fruit extract significantly affected the

induction of increased SOD in hyperglycemic rats. The K45 treatment was the best treatment for SOD induction. Red fruit extract decrease in blood glucose in hyperglycemia rats. The K45 treatment was the best treatment for SOD induction.

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REFERENCES

- Adetasia, T. (2021). Pengaruh pemberian ekstrak buah merah (*Pandanus conoideus* Lam.) terhadap penurunan kadar kolesterol total dan kadar LDL (low density lipoprotein) pada tikus putih (*Rattus norvegicus*) yang mendapat diet aterogenik. (*Doctoral dissertation*). Wijaya Kusuma Surabaya University.
- Bourebaba, N., Kornicka-Garbowska, K., Krzysztof, M., Bourebaba, L., Kowalczyk, A., (2021). Laurus nobilis ethanolic extract attenuates hyperglycemia and hyperinsulinemia-induced insulin resistance in HepG2 cell line through the reduction of oxidative stress and improvement of mitochondrial biogenesis–Possible implication in pharmacotherapy. *Mitochondrion* 59 (2021): 190-213.
- Berawi, K. N., & Agverianti, T. (2017). Efek aktivitas fisik pada proses pembentukan radikal bebas sebagai faktor risiko aterosklerosis (The effect of physical activity on the process of free radical formation as a risk factor for atherosclerosis). *Jurnal Majority*, 6(2), 86-91.
- Bommer, C., Sagalova, V., Heesemann, E., Manne-Goehler, J., Atun, R., Bärnighausen, T., Davies, J. and Vollmer, S., (2018). Global economic burden of diabetes in adults: projections from 2015 to 2030. *Diabetes care*, 41(5), pp.963-970.
- Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. *Frontiers in chemistry*, 11, 1158198.
- Finley, J. W., Kong, A. N., Hintze, K. J., Jeffery, E. H., Ji, L. L., & Lei, X. G. (2011). Antioxidants in foods: state of the science important to the food industry. *Journal of agricultural and food chemistry*, 59(13), 6837-6846.
- Fadli, D. M. A. (2021). Pengaruh buah merah (*Pandanus conoideus* lam) terhadap kadar HDL (High Density Lipoprotein) pada tikus wistar jantan yang mendapat diet tinggi lemak (The effect of red fruit (*Pandanus conoideus* lam) on HDL (High Density Lipoprotein) levels in male Wistar rats receiving a high-fat diet). (*Doctoral dissertation*, Wijaya Kusuma Surabaya University).
- Gunawan, I. A., Fujii, R., Maoka, T., Shioi, Y., Kamebun, K. M. B., Limantara, L., & Brotosudarmo, T. H. P. (2021). Carotenoid composition in buah merah (*Pandanus conoideus* Lam.), an indigenous red fruit of the Papua Islands. *Journal of Food Composition and Analysis*, 96, 103722.
- Halliwel, B. (2023). Understanding mechanisms of antioxidant action in health and disease. *Nature Reviews Molecular Cell Biology*, 1-21.
- Kesavadev, J., Misra, A., Saboo, B., Aravind, S. R., Hussain, A., Czupryniak, L., & Raz, I. (2021). Blood glucose levels should be considered as a new vital sign indicative of prognosis during hospitalization. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 15(1), 221-227.
- Makrilakis, K., Liatis, S., Tsiakou, A., Stathi, C., Papachristoforou, E., Perrea, D., & Niakas, D. (2018). Comparison of health-related quality of Life (HRQOL) among patients with pre-diabetes, diabetes, and normal glucose tolerance, using the 15D-HRQOL questionnaire in Greece: the DEPLAN study. *BMC endocrine disorders*, 18, 1-10.
- Mahmud, A. R., Ema, T. I., Siddiquee, M. F. R., Shahriar, A., Ahmed, H., Mosfeq-Ul-Hasan, M., Mizan, M. F. R. (2023). Natural flavonols: actions, mechanisms, and potential therapeutic utility for various diseases. *Beni-Suef University Journal of Basic and Applied Sciences*, 12(1), 47.
- Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free radical properties, source and targets, antioxidant consumption and health. *Oxygen*, 2(2), 48-78.
- Mas-Bargues, C., Escriva, C., Dromant, M., Borrás, C., & Vina, J. (2021). Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease. *Archives of Biochemistry and Biophysics*, 709, 108941.
- Papachristoforou, E., Lambadiari, V., Maratou, E., & Makrilakis, K. (2020). Association of glycemic indices (hyperglycemia, glucose variability, and hypoglycemia) with oxidative stress and diabetic complications. *Journal of diabetes research*, 2020.
- Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin. Diabetes Atlas Committee. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes research and clinical practice*, 157, 107843.
- Taylor, S. I., Yazdi, Z. S., Beitelshes, A. L. (2021).

- Pharmacological treatment of hyperglycemia in type 2 diabetes. *The Journal of Clinical Investigation*, 131(2).
- Wabula, R. A., Seniwati, S., & Widiastuti, H. (2019). Aktivitas antioksidan ekstrak etanol buah merah (*Pandanus conoideus* Lam.) dengan metode ferric reducing antioxidant power (FRAP). *Window of Health: Jurnal Kesehatan*, 329-337.
- Willcox, J. K., Ash, S. L., & Catignani, G. L. (2004). Antioxidants and prevention of chronic disease. *Critical reviews in food science and nutrition*, 44(4), 275-295.
- Zaloga, G. P. (2021). Narrative review of n-3 polyunsaturated fatty acid supplementation upon immune functions, resolution molecules and lipid peroxidation. *Nutrients*, 13(2), 662.
- Zebua, L. I., & Walujo, E. B. (2016). Pengetahuan tradisional Masyarakat Papua Dalam Mengenali, Mengklasifikasi dan Memanfaatkan Pandan Buah Merah (*Pandanus conoideus* Lam). *Jurnal Biologi Papua*, 8(1), 23-37.
- Zengin, H. (2021). The effects of feeding and starvation on antioxidant defence, fatty acid composition and lipid peroxidation in reared *Oncorhynchus mykiss* fry. *Scientific Reports*, 11(1), 16716.