Inhibitory Activity of the α-Glucosidase Enzyme by Albumin Isolated from Giant Gourami (*Osphronemus Goramy*), Rice Eel (*Monopterus albus*), and Mackerel Tuna (*Euthynnus affinis*)

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Abstract. The giant gourami (*Osphronemus goramy*), rice eel (*Monopterus albus*), and mackerel tuna (*Euthynnus affinis*) are among the sources of protein and albumin that have been used to lower the blood glucose level of people with Diabetes Mellitus (DM). This study aims to determine the protein levels and albumin content of *O. goramy*, *M. albus*, and *E. affinis* and the inhibition activity of albumin against the α-glucosidase enzyme. This study employed an experimental method with accidental sampling. The protein content of fresh fish fillets was tested using the Kjeldahl method and extracted using a centrifuge to obtain albumin. Albumin levels were determined using a visible spectrophotometer. The inhibitory activity of albumin against α-glucosidase enzyme was tested using an ELISA reader. The results showed a significant difference between freshwater and seawater fish proteins, namely giant gourami of 13.91%, rice eel of 14.41%, and mackerel tuna of 30.55%. The highest albumin content was obtained from mackerel tuna (4.75 ± 0.04 g/100 mL), followed by giant gourami (3.61 ± 0.26 g/100 mL), and rice eel (2.38 ± 0.26 g/100 mL). The albumin showed no significant activity against the α-glucosidase enzyme.

Keywords: Protein · Albumin · α-glucosidase

1 Introduction

Diabetes Mellitus (DM) is one of the most common health problems in developing countries. The International Diabetes Federation states that Indonesia is ranked 6th globally with 10.3 million DM cases and is predicted to increase to 16.7 million by 2045 [1].

DM is frequently associated with a decrease in albumin levels in the blood. Albumin is a circulating protein synthesized in the liver and accounts for 60% of the total serum protein. In addition to representing a major determinant of oncotic pressure, albumin also serves as a carrier for many endogenous and exogenous compounds, including free fatty acids, ions, and drugs. Clinically, albumin is an important biomarker for assessing liver function, and insulin administration is needed to prevent hypoalbuminemia [2].

Amino acids are other non-glucose ingredients that stimulate insulin release through different mechanisms. Among the amino acids that can stimulate insulin secretion and

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lower blood glucose by inhibiting the activity of the α-glucosidase enzyme are leucine, arginine, lysine, alanine, phenylalanine, isoleucine, and methionine [3, 4]. In addition, proteins are polyamides generated from the hydrolysis of amides to produce carboxylic acids and amines. The amide bonds that bridge two or more amino acids are peptide bonds.

The aquatic biota that has been evident to solve the problem of diabetes is the snakehead. The albumin extract of snakehead fish reduces blood glucose levels in vivo and helps the regeneration cells in the islets of Langerhans [5]. The fish hydrolysate also contains insulin-stimulating amino acids [4].

The continuous exploration of snakehead fish potentially reduces their population while the farming of snakehead fish encounters various problems and diseases that threaten their survival. It urges many researchers to examine other species as the alternative to snakehead fish. The screenings of the albumin and protein profiles of giant (*Osphronemus goramy*) and rice eel (*Monopterus albus*) [6] and mackerel tuna (*Euthynnus affinis*) [7] have been carried out. The results revealed they have high levels of protein and albumin.

Susilowati [8] suggested that based on the Fish Serum Albumin (FSA), giant gourami is potential nutraceutical material with higher albumin levels than snakehead fish. Meanwhile, the extract of rice eel can reduce Tumor Necrosis Factor (TNF) expression and increase epidermal thickness in mice [9]. Moreover, the amino acids in tuna, including histidine, methionine, tyrosine, and lysine, demonstrate strong antioxidant activity [10].

Therefore, the present study also examined the inhibitory activity of the albumin isolated from freshwater fish of giant gourami (*O. goramy*) and rice eel (*M. albus*), and marine fish of mackerel tuna (*E. affinis*) against the α-glucosidase enzyme.

2 Materials and Method

2.1 Materials

The fresh fish were obtained from the Mangu market, Ngesrep, Boyolali, Indonesia. The sampling technique was a simple random technique. The equipment consisted of centrifuge, UV-Vis spectrophotometer, Kjeldahl apparatus, analytical balance, pH meter, magnetic stirrer, and glassware. The chemicals used in the test were Bovine Serum Albumin (BSA), phosphate buffer, ether, sodium sulfite, Folin–Ciocalteu, NaOH, Na_2CO_3, $CuSO_4$, distilled water, Selenium powder, concentrated H_2SO_4, catalyst, $Na_2S_2O_7$, H_3BO_3, and HCl.

2.2 Albumin Isolation

The fresh fish was cleaned thoroughly and filleted. The fish fillets were chopped and blended. A portion of 10 g was weighed, and 25 mL of phosphate buffer solution pH 6.8 was added. It was centrifuged for 20 min at 10.000 rpm. Subsequently, a clear solution (containing protein) was collected and added with 2 mL of 25% sodium sulfite and 2 mL of ether. The yield was centrifuged again. The top layer was removed, while the bottom layer containing albumin was collected.

2.3 Protein Level Analysis

In this study, the method to determine the total protein content of fish was the Kjeldahl method [11].

The nitrogen and protein content of the samples were determined using the formula:
%Total nitrogen = {(vol of titrant x N HCl x 14.007)/sample weight} x 100%
Crude protein content (%) = total nitrogen x 6.25.

2.4 Albumin Level Analysis

The determination of albumin levels was done using the Lowry method [12]. The measurement utilized a UV-Vis spectrophotometer. The absorbance is directly proportional to the albumin concentration of the sample.

Reagents:

1) Reagent A: 2% Na_2CO_3 + 0.1 mol/L NaOH solution.
2) Reagent B: 5% $CuSO_4$ + 1% Na/K tartrate solution.
3) Reagent C: 50 mL of reagent A + 1 mL of reagent B.
4) Reagent D: Folin–Ciocalteu (phenol reagent) dissolved in 1:1 distilled water.
5) Standard protein solution: 0.02 mg/mL bovine serum albumin (BSA).

Standard curve:

1) Concentration: 0 (blank), 0.1–1 mL of protein standard was put into a 10.0 mL volumetric flask. Add water until the total volume of each dilution was 4 mL.
2) 5.0 mL of reagent C was added to each test tube, stirred well and left for 10–15 min at room temperature.
3) Add 0.5 mL of reagent D, stir well and store for about 30 min until the dilution turned blue. Add distilled water to the limit mark.
4) Absorbance was measured at 700 nm. Create a standard curve.

Sample:

Determine the sample concentration, put it into a 10.0 mL volumetric flask, and treat it the same way as the standard curve.

2.5 Inhibitory Activity Test Against α-Glucosidase Enzyme

The in vitro assay of albumin inhibitory activity against α-glucosidase enzyme was tested to inhibit the breakdown of p-nitrophenyl-α-D-glucopyranose into the p-nitrophenyl substrate by the α-glucosidase enzyme. The α-glucosidase enzyme inhibitory activity was measured based on the color of the reaction using an ELISA reader at 400 nm. The reaction mixtures consisted of 10μL blank, control, sample blank, and sample control (giant gourami, rice eel, and mackerel tuna albumin). Subsequently, 20 μL potassium phosphate buffer 0.1 M with pH 7.0 and 10 μL p-nitrophenyl-α-D-glucopyranose substrate 0.5 mM were added to each mixture and then incubated at 37 °C for five minutes. The observation was done on the reaction of the mixtures added with 10μL α-glucosidase

enzyme 0.15 U/mL (Sigma-Aldrich) at 37 °C for 15 min. The reaction was terminated by adding 200 μL sodium carbonate 200 mM.

The inhibition of the α-glucosidase enzyme was calculated using the following equation expressed in percentage [13]:

$$\text{Inhibition } (\%) = [(A1 - A2)/A1] \text{ x } 100\% \quad (1)$$

where:

A1 = The absorbance of control minus the absorbance of blank
A2 = The absorbance of the control sample minus the absorbance of the blank sample

2.6 Data Analysis

The data on the percentage of protein, albumin, and α-glucosidase enzyme inhibitory activity were analyzed using SPSS version 22. The normality test of the data was carried out using the Shapiro-Wilk test, while the homogeneity test was analyzed using the Levene test. The results revealed that the data were normally distributed and homogeneous ($P > 0.05$). Subsequently, the one-way ANOVA parametric test with a 95% confidence level ($\alpha = 0.05$) was conducted, showing significantly different results. Subsequently, the post hock test using the LSD test with a value of 0.05 was done.

3 Results and Discussion

Each fish has different chemical composition depending on the type of fish. The difference also occurs between the individuals of a species and between the parts of a body. It is caused by several factors, including age, metabolic rate, fish movement, food, and reproductive period [6]. Huang reported that the chemical composition of fish might vary depending on species, age, sex, availability of feed-in water, habitat, and environmental conditions [14]. The proximate test results of giant gourami, rice eel, and mackerel tuna are shown in Table 1. This proximate analysis was carried out to identify fish fillet samples' protein, fat, moisture, ash, and carbohydrate composition.

The average moisture content of the fish fillet samples was above 70%. The analysis of variance showed that the samples were significantly different ($\alpha = 0.05$). The moisture content analysis revealed the highest water content in rice eel (78.42%), while the lowest was in tuna (71.55%).

The ash content represents the mineral content of the fish fillet. The data in Table 1 show that the mineral content of the samples was relatively low. The analysis of variance uncovered that there was a significant difference between samples ($\alpha = 0.05$).

The average fat contents of giant gourami, rice eel, and mackerel tuna were 1.94%, 0.09%, and 3.10%, respectively. The analysis of variance showed that the reactions between the treatments were significantly different ($\alpha = 0.05$). In this case, the fat content of fish fillets usually causes rancidity in the storage process [12].

Table 1. Proximate Test of Fish Fillet Samples

Sample	Analysis Parameters			
	Moisture (%Bw)	Ash (%Bw)	Fat (%Bw)	Protein (%Bw)
Giant Gourami (*O. Goramy*)	74.49	0.6	1.94	13.91
Rice Eel (*M. Albus*)	78.42	1.28	0.09	14.41
Mackerel Tuna (*E. Affinis*)	71.55	2.21	3.10	30.55

The protein content of the samples was relatively high, as shown in Table 1. It indicates that giant gourami, rice eel, and mackerel tuna are food sources with high nutritional value. Protein can be used as an energy source other than carbohydrates and fats. Proteins also act as enzymes that form antibodies and bind to other molecules to form a complex molecule. In addition, fish is a complete protein containing both essential and non-essential amino acids. Amino acids are very useful for muscle protein synthesis. The protein cycle occurs in cells, tissues, and other body parts and involves the digestive tract.

According to Fallah research [15], habitat can affect the chemical content of the fish. This study used giant gourami and rice eel representing freshwater fish and mackerel tuna representing marine fish. Table 1 shows that the protein content of marine fish (mackerel tuna) was 30.55%, greater than the protein content of freshwater fish (giant gourami and rice eel of 14.41% and 13.91%, respectively). The factors that affect the protein content are pH level and the distribution of microorganisms in a habitat [16].

The ANOVA test showed that the protein content of giant gourami and rice eel was not significantly different, of 0.376 ($p < 0.05$). Meanwhile, the protein of mackerel tuna was significantly different from other samples. It illustrates that the difference in habitat affected the protein content of fish.

The correlation between BSA standard and analyte absorbance, close to 1, indicates the test's high sensitivity, as shown in Fig. 1. It also demonstrates that the instrumentation and detector could identify the increase in analyte concentration. The test results showed that all the samples contained albumin and protein.

The albumin profile can be seen in Table 2. ANOVA test of albumin profile of the samples showed a significant difference in albumin content of the fish ($p < 0.05$).

Moreover, patients with type 2 DM who consumed lean fish showed a decreased risk by almost 30% compared to patients without such a diet [17]. Meanwhile, patients with type 2 DM who showed the symptoms of severe hyperglycemia would also experience a significantly decreased albumin concentration in the liver [18].

Albumin acts as a balancing substance in the body, particularly by balancing any residual charge with its relatively large molecules in plasma. Under normal pH conditions, albumin has a negative charge and is useful in the process of forming anion groups that affect acid-base conditions. A decrease in certain albumin concentrations may lead to metabolic alkalosis [19].

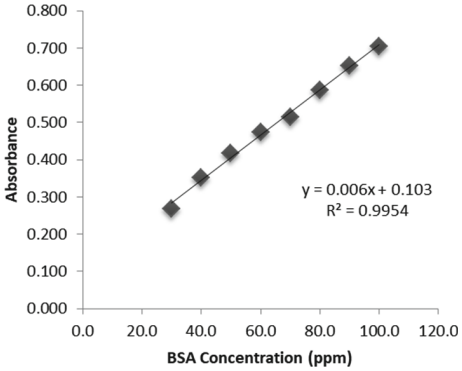

Fig. 1. Bovine Serum Albumin (BSA) Standard Curve

Table 2. The albumin profile of fresh fish samples

Sample	Albumin Level (g/100 mL)
Giant Gourami (*O. Goramy*)	3.61 ± 0.26
Rice Eel (*M. Albus*)	2.38 ± 0.26
Mackerel Tuna (*E. Affinis*)	4.75 ± 0.04

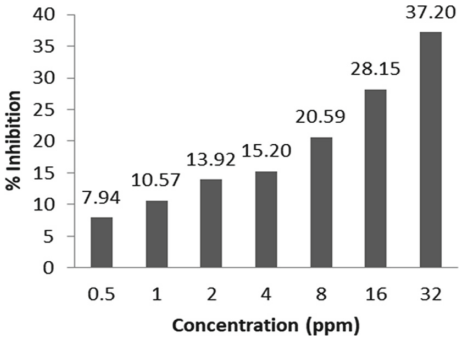

Fig. 2. Comparative graph of acarbose concentration and α-glucosidase inhibition (%)

Further, the inhibition of reactions catalyzed by enzymes can block major metabolic pathways by preventing the formation of essential or unwanted metabolites. The compounds that can inhibit the activity of α-glucosidase enzymes generally show antidiabetic activity. The inhibition of α-glucosidase enzyme activity in DM patients is useful for lowering blood glucose levels, especially after eating [3]. The α-glucosidase enzyme is an isoamylase enzyme that breaks down amylose/amylopectin by cutting the 1,4 glycosidic bonds and the 1,6 glycosidic bonds to produce simple sugars [20]. The inhibition of the α-glucosidase enzyme can reduce the absorption of carbohydrates from food by the intestine, thereby preventing an increase in blood glucose levels.

Figure 2 displays the result of the α-glucosidase enzyme inhibitory activity test using acarbose. It shows that increased concentration is followed by an increased inhibitory activity. The generated equation is $y = 0.8833x + 11.068$ with a correlation value of $R^2 = 0.9455$. The compound test shows that the percentage of inhibitory activity against the α-glucosidase enzyme represents the level of antidiabetic activity [21].

The results of the in vitro inhibition of albumin isolated from giant gourami, rice eel, and mackerel tuna against the α-glucosidase enzyme are shown in Figs. 3, 4, and 5. The comparison used as an inhibitor of the α-glucosidase enzyme was acarbose. Acarbose acts as a competitive antihyperglycemic agent, reversibly inhibiting α-amylase in the pancreas and binding to α-glucosidase enzymes in the intestinal membrane. The mechanism of this drug is to delay the hydrolysis of carbohydrates, disaccharides, and glucose absorption and inhibit the metabolism of sucrose to glucose and fructose [22].

Based on the test, neither giant gourami, rice eel, nor mackerel showed any inhibitory activity. The higher the concentration of the albumin, the lower the percentage of inhibition of the α-glucosidase enzyme. It suggests that albumin had no indication as an antidiabetic agent based on the α-glucosidase inhibitory mechanism.

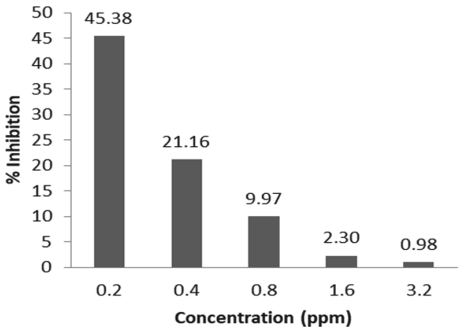

Fig. 3. Comparative Graph of Giant Gourami Albumin Concentration and α-glucosidase Inhibition (%)

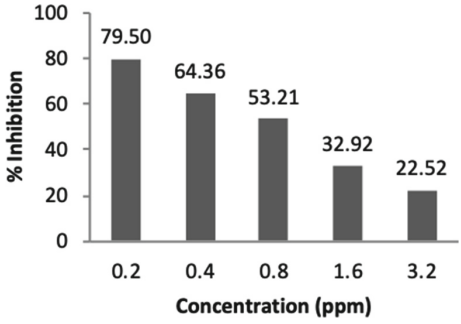

Fig. 4. Comparative Graph of Rice Eel Albumin Concentration and α-glucosidase Inhibition (%)

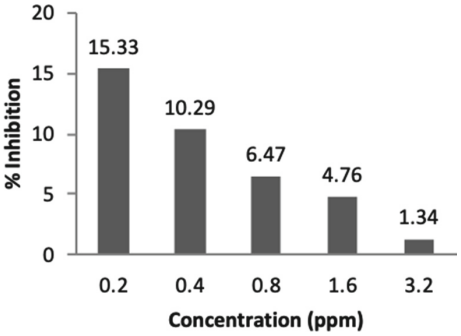

Fig. 5. Comparative Graph of Mackerel Tuna Albumin Concentration and α-glucosidase Inhibition (%)

According to Duttaroy [23], albumin displays a characteristic of a strong long-acting insulin analog that can be evaluated for a novel therapy for patients with insulin-dependent diabetes. Insulin stimulates peripheral glucose disposal and inhibits hepatic glucose production. Pretreatment of cells with insulin or albumin results in a dose-dependent increase of 2-deoxyglucose uptake with very similar characteristics. Albumin is as potent as recombinant human insulin in inhibiting gluconeogenesis.

Albumin compounds are also reported to have antioxidant activity through the mechanism of action of multiple-binding sites on free radicals. These antioxidants will reduce the rate of lipid peroxidation in damaged beta cells and regenerate them [24]. In addition, the pancreas is used as a vital parameter in regulating glucose in the blood system. The key role of pancreatic cells is to secrete insulin. Abdulgani stated that albumin derived from snakehead (*C. striata*) could improve the islets of Langerhans. It was demonstrated by an administration of a therapeutic dose of 0.14846 mL in rats (previously induced with alloxan) that showed an improvement in the islets of Langerhans by 50.10 μm. The increase in the average diameter of the islets of Langerhans between positive controls and the therapeutic dose was 68.78% [5]. Therefore, albumin may be used as an antidiabetic agent through other mechanisms. Thus, further research is needed to examine its pharmacological effects.

4 Conclusion

The results of this study showed that there was a significant difference between freshwater and seawater fish. Giant gourami had protein of 13.91%, rice eel had protein of 14.41%, and mackerel tuna had protein of 30.55%. The highest albumin concentration to the lowest was mackerel tuna 4.75 ± 0.04 g/100 mL, giant gourami 3.61 ± 0.26 g/100 mL, and then rice eel 2.38 ± 0.26 g/100 mL. The albumin had no significant activity against the α-glucosidase enzyme.

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Authors' Contributions. All authors helped shape the research, analyzed the results, and contributed to the final manuscript.

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