Protein Profiles of Snakehead (*Channa striata*), Catfish (*Pangasius hypopthalmus*), and Mackerel (*Rastrelliger spp.*) and Their Effectiveness as Antidiabetic Agents

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Abstract. The safety of oral antidiabetic drugs as the first-line therapy for Diabetes Mellitus (DM) has been a prevailing issue. Meanwhile, these drugs are used for long-term treatment and they often require combination therapy. Natural medicine is an alternative to address the safety issue of drugs because of its minimal side effects and abundant availability. One of the alternatives is snakehead (*Channa striata*), which has been reported to have antidiabetic activity derived from the regeneration of the damaged pancreatic islets of Langerhans. In addition, snakehead fish is a source of amino acids that lower the accumulation of blood glucose. This study aimed to examine the protein profiles and antidiabetic activity of catfish (*Pangasius hypopthalmus*) and mackerel (*Rastrelliger spp*) and to determine their capacities as alternatives to snakehead. The albumin measurements showed that the albumin levels of snakehead, catfish, and mackerel were 4.84 ± 0.54%, 2.57 ± 0.29%, and 2.39 ± 0.29%, respectively. The proximate analysis showed a protein content of 11.8%, 13.58%, and 16.93%, respectively. The screening of α-glucosidase inhibitory activity obtained that the inhibitory activities of the samples were lower than that of acarbose as the control ($p < 0.05$). Interestingly, all the samples indicated a similar pattern in α-glucosidase inhibitory activity, of which the hydrolysate had a higher potency than the albumin, and the albumin was higher than the powder content ($p < 0.05$). Briefly, catfish and mackerel can be the alternatives to snakehead to support the management of DM therapy.

Keywords: Channa striata · Pangasius hypopthalmus · Rastrelliger spp · α-glucosidase

1 Introduction

Diabetes mellitus (DM) is a major health problem worldwide and is associated with the increased mortality rate, decreased quality of life, and high costs of therapy [1]. The International Diabetes Federation (IDF) predicted that 425 million people were living

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with DM in 2017 and projected 45% growth (629 million people by 2045), specifically in the Asia Pacific region (159 million people in 2017) and an increase of 15% (183 million people by 2045) [2].

Today, oral antidiabetic drugs remain the first-line therapy for the treatment of type 2 DM. Moreover, the treatment is a long-term one that requires combination therapy with more than one drug. Therefore, the issues related to the tolerance and safety of antidiabetic drugs are crucial [3]. The management of DM with minimal side effects is still a challenge for health practitioners. Natural medicine is considered safer than synthetic medicine due to its minimal side effects and it is more accessible without prescription [4]. One of the alternative treatments for DM involves snakehead (*Channa striata*), which has been reported to possess an antidiabetic activity that lower blood glucose levels [5]. It occurs due to the regeneration of the damaged pancreatic islets of Langerhans by the snakehead albumin [5]. In addition, snakehead fish is a source of amino acids that can reduce the buildup of glucose in the blood [6] by inhibiting the activity of the α-glucosidase enzyme. These amino acids include leucine, arginine, lysine, alanine, phenylalanine, isoleucine, and methionine. [7]. The quality of the essential and non-essential amino acids in snakehead fish is superior to eggs [8]. Despite the increasing demand for snakehead fish, its natural population is decreasing [9]. The current fish farming also experiences various problems, including the threat of various types of diseases [10]. Therefore, exploration of the alternatives for snakehead as the source of albumin and amino acids is required. Catfish (*Pangasius hypopthalmus*) have a relatively higher albumin and protein content than other types of fish [11]. Meanwhile, mackerel (*Rastrelliger spp*) has a high protein content and is one of the most consumed fish in Indonesia [12].

This study aimed to identify and compare the albumin level and amino acids in catfish, mackerel, and snakehead, and to probe their antidiabetic activities through in vitro α-glucosidase inhibitory assay.

2 Method

2.1 Equipment and Materials

The equipment used in this study included UV-Vis Spectrophotometer Shimadzu UV-1280, Elisa Epoch Reader 2104198, Hettich EBA 200 centrifuge, Oregon LC-04S centrifuge, freeze dryer, Ohaus analytical balance, Memmert UN55 incubator, Eppendorf micropipette 10-100μl and 100-1000μl, Eppendorf multichannel pipette, Hellma Analytics 10mm cuvette, Corning Costar 96 well plate, blender, 60 mesh sieve, glassware, BSA Sigma Aldrich, Folin Ciocalteu, Na_2CO_3, NaOH, $KnaC_4H_4O_6.4H_2O$, $CuSO_4.5H_2O$, KH_2PO_4, trypsin enzyme, p-Nitrophenyl-α-D-glucopyranoside, and α-glucosidase enzyme. The samples of catfish, mackerel, and snakehead were procured from Balekambang Fish Market, Surakarta, Indonesia.

2.2 Fish Sample Preparation

The fins, skins, bones, and internal organs of the fresh catfish, mackerel, and snakehead from Balekambang Fish Market were removed. The fish fillets were divided into two

parts: one part was stored for albumin content and proximate analysis, while the other was oven-dried at a temperature of 40 °C. The dried fish fillets were pulverized and passed through a sieve to obtain 60 mesh size powder. The powder was used in protein hydrolysis to produce the fish protein hydrolysate. Then, hydrolysate was tested for amino acid content identification.

2.3 Albumin Level Test

The isolation of albumin was conducted using a method in Sari et al. [13] with modifications. Firstly, 25 ml of phosphate buffer (pH 6.8) was added to 10g of fresh fish fillet. The mixture was centrifuged at 6000 rpm for 20 min. The supernatant was added with 2 ml of 25% sodium sulfite and 2 ml of ether. The mixture was centrifuged at 6000 rpm for 20 min. The bottom layer (albumin) was collected [13]. The test of albumin level was performed using the Lowry method described by Fatma et al. with some adjustments [14].

2.4 Identification & Test of Amino Acid Levels

Protein hydrolysis was carried out according to the method described by Karnila [7] after modifications. The suspension of 4 g of fish powder in 100 ml of distilled water was followed by homogenization for 2 min. Subsequently, the suspension was boiled at 98 °C for 15 min. After cooling down, 880 μg/ml of suspension was mixed with trypsin enzyme. The hydrolysis was done at 37 °C and pH 7.5 for 24 h. The yield was boiled at 85 °C for 15 min for enzyme inactivation. Subsequently, the supernatant (liquid phase) was separated from the precipitate (residue) through centrifugation at 6000 rpm for 15 min at 4 °C. The supernatant obtained from the separation was subjected to freeze drying [7]. The hydrolysate was examined for the identification of amino acids and their concentrations using LC-MS/MS and UPLC-MS/MS.

2.5 Proximate Analysis

Proximate analysis was conducted using the method suggested by AOAC[15]. The moisture content, minerals, total protein, total fat, and carbohydrates were calculated.

2.6 α-Glucosidase Inhibitory Activity Test

The in vitro α-glucosidase enzyme inhibition activity was implemented using ρ-nitrophenyl-α-D-glucopyranoside as the substrate, yielding the yellow colored ρ-nitrophenyl and glucose by the α-glucosidase enzyme. The enzyme inhibitory activity was measured based on the color of the reaction at 410 nm with the help of a spectrophotometer. The inhibitory activity of α-glucosidase enzyme was identified using 0.5 mM ρ-nitrophenyl-α-D-glucopyranoside as the substrate. An amount of 0.1 M potassium phosphate buffer with pH 7 was prepared. The procedure was to mix the reactions consisting of the blank, control A, control B, and the sample with the combination presented in Table 1.

Table 1. Solution Composition of α-glucosidase Inhibitory Activity Test

	Blank (μL)	Control A (μL)	Control B (μL)	Sample (μL)
Albumin/Hydrolysate Sample	0	0	10	10
Buffer	10	10	0	0
Buffer	20	20	20	20
Substrate	10	10	10	10
Incubation 37 °C for 5 min				
Enzyme	0	10	0	10
Na_2CO_3	200	0	200	0
Incubation 37 °C for 15 min				
Enzyme	10	0	10	0
Na_2CO_3	0	200	0	200

The percent of inhibition was determined using Eq. (1), in which A1 is the absorbance of control A minus the absorbance of control blank, and A2 is the absorbance of the sample minus the absorbance of control B [7]. The results of α-glucosidase inhibition assay were statistically analyzed using IBM SPSS statistical viewer version 22.

Equation (1)

$$\frac{A1 - A2}{A1} \text{x } 100\% \tag{1}$$

3 Results and Discussion

The albumin levels of snakehead, catfish, and mackerel found 4.84 ± 0.54%, 2.57 ± 0.29%, and 2.39 ± 0.29%, respectively. In this study, the highest albumin content was obtained in snakeheads, followed by catfish and mackerel. Fish, especially freshwater fish, is a source of albumin and protein for human beings [11].

The proximate test data are shown in Table 2. Based on the data, the highest total protein was found in mackerel, followed by catfish and snakehead, namely 16.93%, 13.58%, and 11.8%, respectively. Protein has a significant role in body functions, such as providing essential amino acids [16]. The identification of amino acids and their concentrations in the three fish hydrolysate samples are presented in Table 3.

The identification of types and levels of amino acids showed that there were 16 types of amino acids in the hydrolysate of snakehead and catfish and 12 types of amino acids in mackerel. They incorporated several amino acids that had been reported to have an antidiabetic effect, namely methionine, phenylalanine, isoleucine, alanine, arginine,

Table 2. Proximate Test

Sample	Analysis Parameters									
	Water (%wb)		Mineral (%wb)		Fat (%wb)		Protein (%wb)		Carbohydrate (%wb)	
Snakehead	77.27	77.35	1.07	1.1	0.2	0.2	11.39	11.8	10.07	9.57
	77.42		1.13		0.19		12.2		9.06	
Catfish	72.96	73.21	0.53	0.55	0.96	0.99	14.33	13.58	11.22	11.68
	73.46		0.56		1.01		12.83		12.14	
Mackerel	71.74	71.97	1.35	1.36	0.12	0.13	17.07	16.93	9.72	9.62
	72.2		1.37		0.14		16.78		9.51	

*wb = wet basis

lysine, and leucine [7]. However, only 5 of the identified 7 amino acids with the antidiabetic effect were detected in mackerel. (no phenylalanine and arginine were detected). The concentration of amino acids varied in each type of fish.

The results of the measurement of α-glucosidase enzyme inhibitory activity are summarized in Table 4. The concentrations of albumin, hydrolysate and powdered samples of each fish were calculated and compared with the positive control using acarbose. The results implied the inhibitory activity in all samples, however less than that of acarbose. The highest inhibitory activity was found in mackerel, followed by catfish and snakehead fish ($p < 0.05$). This corresponds to the amount of total protein content obtained from the proximate test of fish. Since the antidiabetic activities are derived from albumin and amino acids; protein fish can provide high quantity of amino acids and albumin for humans [17]. Meanwhile, based on the types of fish samples, the highest inhibitory activity was demonstrated by hydrolysate, followed by albumin and fish powdered sample ($p < 0.05$). It is plausible due to amino acid content in the hydrolysate that indicates antidiabetic effects through the inhibition of α-glucosidase enzyme [7]. In form of hydrolysate, protein changes into simple form as free amino acids [17]. Therefore, hydrolysate has the highest inhibitory activities than albumin and powdered. Moreover, amino acids can stimulate insulin excretion through various pathways [7]. For example, arginine is an amino acid that improves beta cell function and stimulates energy expenditure as well as insulin sensitivity. Leucine functions in gene transcription and protein synthesis in pancreatic beta cells [18]. Meanwhile, albumin plays as antidiabetic due to its antioxidant properties that can reduce the rate of lipid peroxidation in the damaged beta cells and regenerate them [19]. The concentration of albumin of the samples showed no significant difference ($p > 0.05$).

Table 3. Results of The Identification of Types and Levels of Amino Acids

No	Type of Amino Acids	Concentration (mg/kg)								
		Snakehead			Catfish			Mackerel		
		Simplo	Duplo	Avg	Simplo	Duplo	Avg	Simplo	Duplo	Avg
1	**L-Cystine**	ND	ND	**ND**	ND	ND	**ND**	ND	ND	**ND**
2	**L-Methionine**	505.99	505.8	**505.90**	521.98	519.72	**520.85**	489.17	488.7	**488.94**
3	**L-Serine**	289.02	287.87	**288.45**	278.2	276.42	**277.31**	< 149.74	< 149.74	**< 149.74**
4	**L-Glutamic Acid**	1828.48	1827.95	**1828.22**	1494.95	1496.59	**1495.77**	1054.07	1053.59	**1053.83**
5	**L-phenylalanine**	479.06	476.35	**477.71**	< 476.07	< 476.07	**< 476.07**	ND	ND	**ND**
6	**L-Isoleucine**	354.34	351.17	**352.76**	305.97	305.11	**305.54**	174.5	173.45	**173.98**
7	**L- Valine**	485.98	483	**484.49**	430.41	426.83	**428.62**	263.99	261.78	**262.89**
8	**L-Alanine**	1075.26	1075.04	**1075.15**	956.6	957.62	**957.11**	592.21	593.5	**592.86**
9	**L-Arginine**	< 386.22	< 386.22	**< 386.22**	< 386.22	< 386.22	**< 386.22**	ND	ND	**ND**
10	**Glycine**	374.15	372.72	**373.44**	293.55	292.44	**293.00**	106.36	106.26	**106.31**
11	**L-Lysine**	901.43	900.13	**900.78**	740.78	743.75	**742.27**	892.33	896.27	**894.30**
12	**L-Aspartic Acid**	1514.48	1509.83	**1512.16**	995.6	994.34	**994.97**	543.99	544.07	**544.03**
13	**L-Leucine**	611.05	608.16	**609.61**	513.3	508.83	**511.07**	248.54	245.78	**247.16**
14	**L-Tyrosine**	< 608.01	< 608.01	**< 608.01**	< 608.01	< 608.01	**< 608.01**	ND	ND	**ND**
15	**L-Proline**	232.1	231.03	**231.57**	381.59	382.37	**381.98**	< 128.38	< 128.38	**< 128.38**
16	**L-Threonine**	401.29	399.81	**400.55**	324.79	322.85	**323.82**	< 163.11	< 163.11	**< 163.11**
17	**L-Histidine**	< 295.11	< 295.11	**< 295.11**	< 295.11	< 295.11	**< 295.11**	ND	ND	**ND**

*ND = Not Detected

Table 4. The α-glucosidase enzyme inhibitory activity test

Control		Sample								
		Snakehead			Catfish			Mackerel		
C (ppm)	**Acarbose**	**Ab**	**H**	**P**	**Ab**	**H**	**P**	**Ab**	**H**	**P**
10	14.81	3.63	8.05	3.92	9.02	8.71	4.37	12.94	8.43	4.38
20	18.76	6.79	8.34	4.44	9.50	9.03	4.76	13.74	13.06	4.63
40	19.92	7.22	10.22	7.03	11.65	12.91	5.79	17.74	17.73	6.59
80	23.37	7.85	16.10	7.28	12.47	13.81	7.14	18.42	20.23	6.42
160	30.28	11.38	16.22	8.96	13.77	17.64	7.90	19.07	22.54	7.52
320	32.07	15.89	19.10	11.57	16.00	21.09	12.11	21.22	30.12	8.04

*Ab = albumin, H = hydrolysate, P = powdered

4 Conclusion

The albumin content of snakehead fish was higher than catfish and mackerel. Moreover, 16 types of amino acids were detected in snakehead fish and catfish, while 7 of these amino acids exhibited an antidiabetic effect. Simultaneously, 12 types of amino acids were detected in mackerel and 5 of them also exhibited an antidiabetic effect. The results of the α-glucosidase enzyme inhibitory activity test revealed the inhibitory activity of each sample, that is smaller than that of acarbose. The highest inhibitory activity was demonstrated by mackerel, followed by catfish and snakehead. Given the types of samples, the highest inhibitory activity was demonstrated by hydrolysate, followed by albumin and fish powder. The concentration of the sample did not show a significant effect on the inhibition of α-glucosidase enzyme. Based on the results of this study, it can be determined that catfish and mackerel can be an alternative to snakehead fish as they demonstrate their effectiveness as antidiabetic agents.

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