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Potential of Tilapia Skin Hydrolysate as an Antihypertensive Nutraceutical Using in Silico

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A B S T R A C T

Hypertension sufferers are estimated to continue to increase every year. The prevention process can be done by consuming antihypertensive nutraceuticals. Bioactive peptides are one of the antihypertensive nutraceuticals. This peptide works by inhibiting Angiotensin-converting Enzyme. This peptide is obtained by hydrolyzing tilapia skin using the papain enzyme. This research aims to determine the potential of bioactive peptides from hydrolysis of tilapia skin which act as ACE inhibitors. This research was carried out using the in silico method. The software used is BIOPEP, Peptide Ranker, ProTox III, and SwissADME. The results of the BIOPEP analysis showed 6 groups of peptide activity, dominated by ACE inhibitors and DPP IV inhibitors. The result of Peptide Ranker is that tilapia skin hydrolyzate has a high bioactivity content. Tilapia skin hydrolyzate has also been proven to be categorized as less dangerous with toxicity levels, especially in classes IV and V. The results of SwissADME are that the peptide has good gastrointestinal absorption and is soluble in water. All peptides in tilapia skin hydrolyzate met Lipinski's rules for druglikeness. Further research must be carried out in vivo to explain the potential content of tilapia skin hydrolyzate which can be used as a bioactive agent for antihypertensive nutraceuticals in peptide form.

Keywords: hypertension, peptides, tilapia skin hydrolysis, in silico, antihypertensive

1. Introduction

Hypertension is one of the main health problems and is still a global health problem today. Hypertension is said to be the leading cause of cardiovascular disease as well as the cause of morbidity in the world. In addition, the prevalence of hypertension is increasing, especially in countries classified as low and middle income countries (LMICs) [1]. Data from the World Health Organization (WHO) in 2015 showed that about 1.13 billion people in the world have hypertension, meaning that 1 in 3 people in the world are diagnosed with this condition. The number of people with hypertension continues to increase every year, it is estimated that by 2025 there will be 1.5 billion people affected by hypertension, and it is estimated that 9.4 million people die every year due to hypertension and its complications. Uncontrolled hypertension results in complications in the heart organ including myocardial infarction, coronary heart disease, and congestive heart failure. If complications of hypertension occur in the brain organs, it can cause a stroke. High blood pressure can cause various serious complications if it is persistent and not treated through therapy.

Antihypertensive drugs are a class of drugs that are widely used to reduce high blood pressure or hypertension. The classification of antihypertensives consists of diuretics, α – blocker receptors (alpha inhibitors), β – blocker receptors (beta inhibitors), calcium antagonists, ACE inhibitors, angiotensin II receptor blockers (ARBs), alpha–2 receptor agonists, peripheral adrenergic inhibitors, and renin inhibitors [2]. Currently, antihypertensive drugs on the market act as ACE inhibitors such as Amlodipine, Bisoprollol, and Captopril are drugs from synthetic chemicals that have side effects [3]. Therefore, another alternative is needed to inhibit ACE, namely by utilizing animal resources, one of which is tilapia shell waste.

Tilapia (*Oreochromis niloticus*) is included in the type of freshwater fish cultivation in Indonesia whose production continues to increase. BKIPM (2019) data on fisheries export statistics shows that the export figure of tilapia products in 2018 reached 10 tons spread across various countries such as the United States, Germany, the Netherlands, and Japan. However, the more the tilapia processing industry improves, the more by-products are produced. More than 60% of the by-products produced from the fish processing industry are waste. The processing of tilapia into fillet products leaves a by-product of tilapia skin with a proportion of 8.7% of the total weight of the fish [4] which has the potential to be used as a source of medicinal preparations from natural ingredients that are known to have fewer side effects than chemically synthesized drug preparations.

Tilapia skin, which is a by-product of the fish processing industry, is known to still contain a lot of protein-rich ingredients so that it has the potential to be a nutraceutical antihypertensive. Research on tilapia skin hydrolysate has been widely explored by previous researchers, such as in the research conducted by [5] and [6]. However, the study has a drawback where it only mentions that tilapia skin hydrolysate is only limited to its potential as an

antihyperglycemic and antidiabetic. Therefore, this study was conducted to determine the potential of bioactive peptides from the hydrolysis of tilapia skin which acts as an antihypertensive nutraceutical using in silico.

2. Materials and Methods

Preparation of Tilapia Skin Hydrolisate Database

The LPGERGRPGAPGP sequence was obtained from a study [5] that used tilapia (*Oreochromis niloticus*) skin. The tools used in this study are laptops with Acer Swift 3 specifications, Windows 11 operating system, and software used, among others, BIOPEP, Peptide Ranker, ProTox III, and SwissADME.

Computational Methods

Prediction of bioactive activity and proteolytic analysis of hydrolysate from tilapia skin (*Oreochromis niloticus*) was carried out using BIOPEP (https://biochemia.uwm.edu.pl/en/biopep-uwm-2/) software [7]. BIOPEP can analyze the potential content of bioactive peptides and examine the sensory characteristics of hydrolysate from tilapia skin (Oreochromis niloticus) with sequences obtained from research [5]. Peptide rank analysis using peptide sequence fragment material was analyzed using Peptide Ranker (http://distilldeep.ucd.ie/PeptideRanker/). Peptide Ranker is a server that can rank peptides according to the level of peptide activity. The Peptide Ranker score is sorted based on the results on peptide bioactivity predictions. Peptide Ranker can predict the probability (between 0 and 1) of a peptide to become bioactive [8]. ProTox III (https://tox.charite.de/protox3/index.php?site=home) is used to determine the toxicity of a peptide compound [9]. SwissADME is a website run by the Swiss Institute of Bioinformatics (SIB) (http://www.swissadme.ch) and is used to predict pharmacokinetic, physico-chemical, lipophilicity, water solubility, medicinal chemistry, and druglikeness properties [10].

3. Results and Discussions

Prediction of Tilapia Skin Hydrolysate Bioactive Peptide Activity (*Oreochromis niloticus*)

The bioactive activity of tilapia skin hydrolysate was analyzed using BIOPEP software. BIOPEP is a bioinformatics software designed to analyze and predict the bioactive potential of peptides from protein sequences. BIOPEP was developed by the Biochemistry Laboratory of Mazury University Poland. The software provides database information in the form of proteins, bioactive peptides, allergenic proteins with their epitopes, sensory peptides, and amino acids. One of the uses of this software is for the evaluation of proteins as precursors of bioactive peptides [11]. BIOPEP makes it possible to analyze protein sequences and find peptide segments that may have bioactive activity. More than 1500 different types of bioactive peptides have been reported and recorded in the BIOPEP database [12]. The software also supports enzymatic process simulation to predict the hydrolysis product of the targeted protein. BIOPEP is an important tool in the research and development of nutraceutical products, pharmaceuticals, and functional foods, as well as assisting scientists and industry professionals in exploring the therapeutic and nutritional potential of bioactive peptides. The peptide activity of tilapia skin hydrolysate can be seen in Table 1.

Table 1. Enzymatic Activity Potential Profile of Peptides from Tilapia Skin Hydrolysate (*Oreochromis niloticus*)

Peptide ID	Bioactive Sequence	Location	Name	Activity	Monoisotopic mass	Chemical mass
2754	PG	[2-3]	Peptide regulating the stomach mucosal membrane activity	Regulating	172.0845	172.1813
2754	PG	[8-9]	Peptide regulating the stomach mucosal membrane activity	Regulating	172.0845	172.1813
3285	PG	[2-3]	Antithrombotic peptide	Antithrombotic	172.0845	172.1813
3285	PG	[8-9]	Antithrombotic peptide	Antithrombotic	172.0845	172.1813
3460	PG	[2-3]	Prolyl endopeptidase inhibitor	Antiamnestic	172.0845	172.1813
3460	PG	[8-9]	Prolyl endopeptidase inhibitor	Antiamnestic	172.0845	172.1813
7625	PG	[2-3]	ACE inhibitor	ACE inhibitor	172.0845	172.1813
7625	PG	[8-9]	ACE inhibitor	ACE inhibitor	172.0845	172.1813

8500	APG	[10-12]	Dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	Dipeptidyl peptidase IV inhibitor		243.1215	243.259
8855	PG	[2-3]	Dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	Dipeptidyl peptidase IV inhibitor		172.0845	172.1813
8855	PG	[8-9]	Dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	Dipeptidyl peptidase IV inhibitor		172.0845	172.1813
9944	ER	[4-5]	ACE inhibitor	ACE inhibitor		303.1538	303.3141
10461	PG	[2-3]	PAM inhibitor	PAM inhibitor		172.0845	172.1813
10461	PG	[8-9]	PAM inhibitor	PAM inhibitor		172.0845	172.1813

P-Proline, G-Glycine, A-Alanine, E-Glutamic acid, R-Arginine

Each peptide analyzed has different activities. Peptide activity can be seen in (Table 1.) which shows the enzymatic activity profile of tilapia skin hydrolysate (Oreochromis niloticus). There were 14 types of peptide bioactive sequences analyzed. Based on Table 1, there are 6 groups of peptide activity dominated by 2 large groups of activity, namely ACE inhibitors (ACEi) and DPP IV inhibitors. ACEi has antihypertensive effects and provides organ protection in common clinical conditions such as diabetes mellitus [13]. DPP IV inhibitor for the treatment of type 2 diabetes mellitus that inhibits the degradation of incretins [14]. Subgroups of peptide activity include regulating, antithrombotic, antiamnestic, and PAM inhibitors. The highest monoisotopic mass was obtained in the ER bioactive peptide sequence with a value of 303.1538 which acted as an ACE inhibitor, while the lowest monoisotopic mass was obtained in the PG bioactive peptide sequence with a value of 172.0845 which acted as a regulating, antithrombotic, antiamnestic, ACE inhibitor, DPP IV inhibitors, and PAM inhibitors. All the activities obtained came from the dipeptide and tripeptide classes. There is a slight difference between monoisotopic mass and chemical mass. BIOPEP can also analyze the sensory in the peptides obtained. Sensory peptides from tilapia skin hydrolysate (Oreochromis niloticus) can be seen in Table 2.

Table 2. Sensory Properties of Peptides from Tilapia Skin Hydrolysate (*Oreochromis niloticus*)

Peptide ID	**Bioactive Sequence**	**Location**	**Name**	**Activity**	**Monoisotopic mass**	**Chemical mass**
1	R	[7-7]	Bitter amino acid	Bitter	174.1114	174.2004
4	P	[13-13]	Bitter amino acid	Bitter	115.0631	115.1301
172	L	[1-1]	Bitter amino acid	Bitter	131.0943	131.1724
270	G	[6-6]	Sweet amino acid	Sweet	75.0319	75.0664
271	P	[13-13]	Sweet amino acid	Sweet	115.0631	115.1301
371	R	[7-7]	Bitterness suppressing amino acid	Bitterness suppressing	174.1114	174.2004
481	PG	[2-3]	Bitter peptide	Bitter	172.0845	172.1813
481	PG	[8-9]	Bitter peptide	Bitter	172.0845	172.1813

P-Proline, G-Glycine, R-Arginine, L-Leucine

Based on Table 2, there are 8 types of peptides analyzed using BIOPEP that contribute to the sensory properties of tilapia skin hydrolysate (*Oreochromis niloticus*). Peptides have a biological role as well as the influence of product taste. Sensory peptides that affect taste have been the object of research for the past 50 years due to their taste-altering properties, including in silico studies of their bitter taste [11]. The number of sensory identification of peptides varies, ranging from 1 to 481 indicating that there are many peptides contributing to the sensory profile. Sensory in the form of taste is classified into three groups consisting of bitterness, sweetness, and bitterness suppressing. Each group has a different taste. The taste was dominated by bitterness with 5 types of peptides contributing, followed by sweetness with 2 types of peptides contributing, and bitterness suppressing with 1 type of peptide contributing. The highest monoisotopic mass is 174.1114 which contributes to the bitter taste and the lowest monoisotopic mass is 75.0319 which contributes to the sweet taste. There is a slight difference between chemical mass and monoisotopic mass. The highest chemical mass is 174.2004 (bitter), while the lowest chemical mass is 75.0664 (sweet). The rating of tilapia skin hydrolysate peptide (*Oreochromis niloticus*) can be seen in Table 3.

Table 3. Peptide Rank of Tilapia Skin Hydrolysate (*Oreochromis niloticus*)

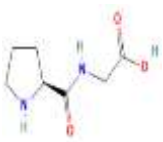
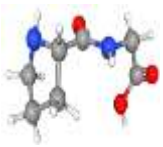
Peptide Rank	**Bioactive Sequence**

0.877086	PG
0.745179	APG
0.0704548	ER

P-Proline, G-Glycine, A-Alanine, E-Glutamic acid, R-Arginine

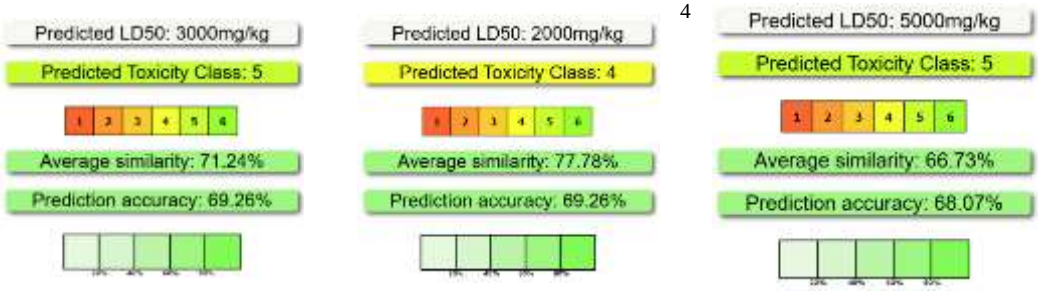
Based on Table 3, it can be seen that there are 3 levels of bioactive peptides found in tilapia skin hydrolysate where there are 12 PG, 1 APG, and 1 ER. The lowest value was obtained by the ER sequence with a value of 0.0704548, while the highest value was obtained by the PG sequence with a value of 0.877086 (majority of peptides). The bioactivity potential of bioactive peptides was identified using the Ranker Peptide tool [15]. The peptide ranker value indicates the probability of bioactivity of each peptide. A higher ranker peptide score indicates a higher content of its bioactive peptides. The threshold value for peptide bioactivity is 0.5. The higher the value means that the peptide has strong bioactivity, while the lower the value (especially below the threshold of 0.5) indicates that there is low or no peptide bioactivity [16]. Identification of peptide chemical profiles can be seen in Table 4.

Table 4. Identification of Chemical Profile of Tilapia Skin Hydrolysate Peptide (*Oreochromis niloticus*)

Bioactive Sequence	**Formula**	**IUPAC**	**Canonical SMILES**	**2D Structure**	**3D Structure**
PG	$C_7H_{12}N_2O_3$	L-prolyl-glycine	C1CC(NC1)C(=O)NCC(=O)O		
APG	$C_{10}H_{17}N_3O_4$	L-alanyl-L-prolyl-glycine	CC(C(=O)N1CCCC1C(=O)NCC(=O)O)N		
ER	$C_{11}H_{21}N_5O_5$	L-alpha-glutamyl-L-arginine	C(CC(C(=O)O)NC(=O)C(CCC(=O)O)N)CN=C(N)N		

P-Proline, G-Glycine, A-Alanine, E-Glutamic acid, R-Arginine

Then the prediction of the toxicity of peptide compounds analyzed using ProTox III can be seen in Figure 1.



PG APG 4 ER

Figure 1. Toxicity of Tilapia Skin Hydrolysate Peptide (*Oreochromis niloticus*)

P-Proline, G-Glycine, A-Alanine, E-Glutamic acid, R-Arginine

Figure 1. shows the predicted toxicity of peptides from tilapia skin hydrolysate (*Oreochromis niloticus*) analyzed using ProTox III. Based on the results obtained, peptides are divided into 2 groups based on toxicity classes consisting of classes IV and V. There are 2 peptides classified as class V with LD50 values of 2000<LD50⩽5000 mg/kg. That is, in small doses it is not deadly, but if consumed continuously or on a large scale it can be dangerous. There

is 1 peptide classified as class IV (dangerous if ingested) with an LD50 value of 300< LD50⩽2000 mg/kg. Peptides classified as Class IV (somewhat harmful) by ProTox III have high LD50 values (2000 mg/kg) indicating that these compounds are not likely to cause significant toxic effects at commonly occurring doses [17]. A higher number of toxicity classes is categorized as less toxic to non-toxic than the lower toxicity class. The pharmacokinetic and bioavailability parameters of tilapia skin hydrolysate peptides analyzed using SwissADME can be seen in Table 5.

Table 5. Pharmacokinetics and Bioavailability of Tilapia Skin Hydrolysate Peptides (*Oreochromis niloticus*) Using SwissADME

Name of Peptide	GI Absorption	BBB Permeant	Bioavailability Score	P-gp substrate	Log K_p (cm/s)
L-prolyl-glycine (PG)	High	No	0.55	No	-10.06 cm/s
L-alanyl-L-prolyl-glycine (APG)	High	No	0.55	No	-10.17 cm/s
L-alpha-glutamyl-L-arginine (ER)	Low	No	0.55	No	-11.67 cm/s

P-Proline, G-Glycine, A-Alanine, E-Glutamic acid, R-Arginine, GI-Gastrointestinal, BBB-Blood-Brain Barrier

Table 5 shows the pharmacokinetic properties and bioavailability of tilapia skin hydrolysate peptide (*Oreochromis niloticus*). There are two peptide compounds, namely PG and APG, which have high digestive absorption, showing good oral bioavailability potential. Then there is one peptide compound, namely ER which has low digestive absorption, indicating that the peptide has poor absorption in the digestive tract and has a low BBB (Blood-Brain Barrier) so it is not effective for treatment in the brain. Bioavailability is used as a consideration for the development of nutraceutical products because bioavailability determines how effectively and efficiently the active ingredients in peptides can be absorbed and used by the body [18]. The higher the bioavailability value, the better it is to be used as a nutraceutical material. The physico-chemical properties of tilapia skin hydrolysate peptides analyzed using SwissADME can be seen in Table 6.

Table 6. Physico-Chemical Properties of Tilapia Skin Hydrolysate Peptide (*Oreochromis niloticus*) Using SwissADME

Name of Peptide	MW	HA	AHA	RB	HBA	HBD	MR	TPSA
L-prolyl-glycine (PG)	172.18	12	0	4	4	3	45.14	78.43
L-alanyl-L-prolyl-glycine (APG)	243.26	17	0	6	5	3	62.56	112.73
L-alpha-glutamyl-L-arginine (ER)	303.31	21	0	11	7	6	73.34	194.12

MW-Molecular Weight (g/mol), HA-Number Heavy Atoms, AHA-Number Aromatic Heavy Atoms, RB-Number Rotatable Bonds, HBA-Number Hydrogen Bond Acceptor, HBD-Number Hydrogen Bound Donor, MR-Molar Refractivity (m^3/mol), TPSA-Topology Polar Surface Area (Å²)

The physico-chemical properties of tilapia skin hydrolysate peptide (*Oreochromis niloticus*) can be seen in Table 6. These properties are important parameters of a molecule that affect efficacy, safety, and metabolism. In addition, these properties can be used to determine drug similarity using Lipinski's rule [19]. The molecular weight and polarity of L-prolyl-glycine (PG) were 172.18 g/mol and 112.73 $Å^2$, respectively. The molecular weight and polarity of L-alanyl-L-prolyl-glycine (APG) are 243.26 g/mol and 78.43 $Å^2$, respectively. Meanwhile, the molecular weight and polarity of L-alpha-glutamyl-L-arginine (ER) are 303.31 g/mol and 194.12 $Å^2$, respectively. Based on these results, the following compounds have met the optimal criteria of their physico-chemical properties. The lipophilicity characteristics of tilapia skin hydrolysate peptides analyzed using SwissADME can be seen in Table 7.

Table 7. Lipophilicity Characteristics of Tilapia Skin Hydrolysate Peptide (*Oreochromis niloticus*) Using SwissADME

Name of Peptide	Log $P_{o/w}$ (iLOGP)	Log $P_{o/w}$ (XLOGP3)	Log $P_{o/w}$ (WLOGP)	Log $P_{o/w}$ (MLOGP)	Log $P_{o/w}$ (SILICOS-IT)	Consensus Log $P_{o/w}$
L-prolyl-glycine (PG)	0.81	-3.81	-1.44	-1.03	-0.40	-1.17
L-alanyl-L-prolyl-glycine (APG)	1.15	-3.36	-1.86	-1.38	-1.17	-1.32
L-alpha-glutamyl-L-arginine (ER)	0.17	-4.96	-2.20	-1.50	-1.74	-2.04

P-Proline, G-Glycine, A-Alanine, E-Glutamic acid, R-Arginine

Table 7. showing the lipophilicity (Log $P_{o/w}$) of tilapia skin hydrolysate peptides. Lipophilicity indicates the octanol or water partition coefficient that predicts the distribution of compounds between the lipophilic (fat) and hydrophilic (water) phases. This value affects absorption and distribution [20]. A high Log P value indicates that the compound is more lipophilic (more fat-soluble), while a low Log P value indicates that the compound is more

hydrophilic (more soluble in water). Based on Table 7. all peptides have low Log P values, indicating that peptides are more soluble in water. The water solubility characteristics of tilapia shell hydrolysate peptides analyzed using SwissADME can be seen in Table 8.

Table 8. Water Solubility Characteristics of Tilapia Skin Hydrolysate Peptide (*Oreochromis niloticus*) Using SwissADME

Name of Peptide	**Log S**	**Water Solubility**		**Class**
		mg/ml	**mol/l**	
L-prolyl-glycine (PG)	1.76	9.83e+03	5.71e+01	Sangat larut
L-alanyl-L-prolyl-glycine (APG)	1.16	3.55e+03	1.46e+01	Sangat larut
L-alpha-glutamyl-L-arginine (ER)	2.13	4.09e+04	1.35e+02	Sangat larut

P-Proline, G-Glycine, A-Alanine, E-Glutamic acid, R-Arginine

Table 8. shows the water solubility characteristics of tilapia skin hydrolysate peptide (*Oreochromis niloticus*) using SwissADME. SwissADME includes a special section to predict water solubility. Water solubility provides important information regarding how well a compound can dissolve in water. Solubility in water is a key parameter in drug design because it affects the absorption, distribution, metabolism, and excretion (ADME) of the compound [21]. Based on Table 8. All peptide compounds have a water solubility class that is highly soluble. Compounds with good water solubility are more easily absorbed in the digestive tract. The solubility of water can also affect the distribution of compounds in the body. Compounds that are more soluble in water are usually easier to circulate in the blood and are easier to excrete through the kidneys. The medicinal chemistry characteristics of tilapia skin hydrolysate peptides analyzed using SwissADME can be seen in Table 9.

Table 9. Characteristics of Medicinal Chemistry of Tilapia Skin Hydrolysate Peptide (*Oreochromis niloticus*) Using SwissADME

Name of Peptide	**A**	**B**	**C**	**D**
L-prolyl-glycine (PG)	No; 1 violation: MW<250	0 alert	0 alert	1.91
L-alanyl-L-prolyl-glycine (APG)	No; 1 violation: MW<250	0 alert	0 alert	2.60
L-alpha-glutamyl-L-arginine (ER)	No; 1 violation: Rotors>7	0 alert	2 alerts: imine_1, imine_2	3.27

A - Leadlikeness; B - Pan Assay Interference Structures (PAINS); C - Brenk; D - Synthetic Accessibility Score

Table 9. shows the medicinal chemistry characteristics of tilapia skin hydrolysate peptide (*Oreochromis niloticus*) using SwissADME. The synthetic accessibility score provides an estimate of the difficulty of compound synthesis. Scores range from 1 (very easy to synthesize) to 10 (very difficult to synthesize). Based on Table 9. All molecules of the peptide have scores of 1.91, 2.60, and 3.27 on the Synthetic Accessibility Score, which makes peptide compounds easy and inexpensive to make synthetically [22]. Lipinski druglikeness characteristics of tilapia skin hydrolysate peptides can be seen in Table 10.

Table 10. *Druglikeness Characteristics* of Tilapia Skin Hydrolysate Peptide (*Oreochromis niloticus*) Using the Lipinski *Rule of Five*

Name of Peptide	**Molecular weight < 500 Dalton**	**Hydrogen bond donor < 5**	**Hydrogen bond acceptors < 10**	**Lipophilicity (LogP < 5)**	**Molar refractivity between 40-130**	**Conclusion**
L-prolyl-glycine (PG)	Yes	Yes	Yes	Yes	Yes	Yes
L-alanyl-L-prolyl-glycine (APG)	Yes	Yes	Yes	Yes	Yes	Yes
L-alpha-glutamyl-L-arginine (ER)	Yes	No	Yes	Yes	Yes	Yes

Based on Table 10. all peptides meet Lipinski's rule. The conclusion from Lipinski's druglikeness analysis was obtained that all peptides have a good possibility of becoming a drug. The conditions for a compound to meet Lipinsky's five rules include a molecular mass of less than 500 Daltons, a hydrogen bond donor of less than 5, a hydrogen bond acceptor of less than 10, a lipophilicity (LogP) of less than 5, and a molar refractive activity between 40-130 [23] [24].

4. Conclusions

Current studies suggest that tilapia skin hydrolysate (*Oreochromis niloticus*) may have the potential as an antihypertensive nutraceutical through ACE inhibitor and DPP IV inhibitor based on in silico analysis. Based on sensory analysis of peptides from tilapia skin hydrolysate (*Oreochromis niloticus*), the most dominant sensory attribute is bitterness followed by sweetness. Tilapia skin hydrolysate has also been proven to be categorized as less harmful with toxicity levels, especially in classes IV and V. This peptide has good gastrointestinal absorption and is water-soluble. All peptides meet Lipinsky's rules for druglikeness. Further research must be conducted in vivo to explain the potential hydrolysate content of tilapia skin which can be used as a bioactive agent for antihypertensive nutraceutical in the form of peptides.

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References

[1] Mills, K. T., Stefanescu, A. dan He, J. 2020. The Global Epidemiology of Hypertension. Nature Reviews Nephrology. 16 (4):223-237

[2] Amalia, A. R. dan Usviany, V. 2023. Evaluation of Side Effects of Antihypertensive Drugs in Patients at RSAU Dr. M Salamun for the June 2023 Period. Health Information: Research Journal. 15 (2):1-10.

[3] Rahmat, P. dan Emelia, R. 2022. Pattern of Prescribing Antihypertensive Drugs for Outpatient Hypertension Patients of RSAU Dr. M Salamun. Indonesian Scientific Journal. 2 (1):133-140.

[4] Peranginangin, R, Murniyati, Nurhayati, Rahmad, W. 2006. Collagen Processing from Tilapia Skin. Jakarta (ID): Penebar Swadaya.

[5] Wang, T. Y., Hsieh, C. H., Hung, C. C., Jao, C. L., Chen, M. C., Hsu, K. C. 2015. Fish Skin Gelatin Hydrolysates as Dipeptidyl Peptidase IV Inhibitors and Glucagon-Like Peptide1 Stimulators Improve Glycaemic Control in Diabetic Rats: a Comparison Between Warm and Cold-Water Fish. J Funct.19:330-40.

[6] Liu, W., Wang, X., Yang, W., Li, X., Qi, D., Chen, H., Liu, H., Yu, S., Pan, Y., Liu, Y. dan Wang, G. 2022. Identification, Screening, and Comprehensive Evaluation of Novel DPP-IV Inhibitory Peptides from The Tilapia Skin Gelatin Hydrolysate Produced Using Ginger Protease. Biomolecules. 12 (12):1-13.

[7] Gangopadhyay, N., Wynne, K., O'Connor, P., Gallagher, E., Brunton, N. P., Rai, D. K. dan Hayes, M. 2016. In Silico and in Vitro Analyses of The Angiotensin-I Converting Enzyme Inhibitory Activity of Hydrolysates Generated From Crude Barley (*Hordeum vulgare*) Protein Concentrates. Food Chemistry. 203:367-374.

[8] Mooney, C., Haslam, N. J., Pollastri, G., Shields, D. C. 2012. Towards The Improved Discovery and Design of Functional Peptidas: Common Features of Diverse Classes Permit Generalized Prediction of Bioactivity. Plos One. 7 (10):1-12.

[9] Riyadi, P. H., Romadhon, R., Anggo, A. D., Herawati, V. E. dan Setyastuti, A.I. 2020. PASS and ADMET Analyses for Eight Compounds from Nile Tilapia (*Oreochromis niloticus*) Viscera Waste Hydrolysate as Anti-Inflammatory Nutraceutical. AACL Bioflux. 13 (5):2630-2638.

[10] Riyadi, P. H., Sari, I. D., Kurniasih, R. A., Agustini, T. W., Swastawati, F., Herawati, V. E. dan Tanod, W. A. 2021. SwissADME Predictions of Pharmacokinetics and Drug-Likeness Properties of small Molecules Present in Spirulina platensis. In IOP Conference Series: Earth and Environmental Science. 890 (1):1-12.

[11] Iwaniak, A., Minkiewicz, P., Darewicz, M., Sieniawski, K. dan Starowicz, P. 2016. BIOPEP Database of Sensory Peptides and Amino Acids. Food Research International. 85:155-161.

[12] Sinthary, V. dan Arief, M. J. 2023. Bioactive Peptide from Goat's Milk Casein as a Source of Antimicrobial and Antioxidant. Jurnal Sains dan Kesehatan. 5 (3):444-457.

[13] Montinaro, V. dan Cicardi, M. 2020. ACE Inhibitor-Mediated Angioedema. International Immunopharmacology. 78:1-6.

[14] Cassano, N., Nettis, E., Di Leo, E., Ambrogio, F., Vena, G. A. dan Foti, C. 2021. Angioedema Associated with Dipeptidyl Peptidase-IV Inhibitors. Clinical and Molecular Allergy. 19 (1):1-12.

[15] Pearman, N. A., Ronander, E., Smith, A. M. dan Morris, G. A. 2020. The Identification and Characterisation of Novel Bioactive Peptides Derived from Porcine Liver. Current Research in Food Science. 3:314-321.

[16] Mooney, C., Haslam, N. J., Holton, T. A., Pollastri, G., Shields, D. C. 2013. Peptide Locator: Prediction of Bioactive Peptides in Protein Sequences. Bioinformatics. 29:1120-1126.

[17] Riyadi, P. H., Susanto, E., Agustini, T. W., Arifin, M. H. dan Prasetyo, D. Y. 2023. Bioactive Peptide Prediction of Skipjack Tuna (*Katsuwonus pelamis*) Hydrolysates Using in Silico. ARPN Journal of Engineering and Applied Sciences. 18 (4):1624-1633.

[18] Lena, N., Jamil, A. S., Muchlisin, M. A. dan Almutahrihan, I. F. 2023. Analysis of the Pharmacological Network of Dutch Teak Plants (*Guazuma ulmifolia* Lamk.) As an Immunomodulator. Journal of Islamic Pharmacy. 8 (1):1-6.

[19] Lohohola, P. O., Mbala, B. M., Bambi, S. M. N., Mawete, D. T., Matondo, A. dan Mvondo, J. G. M. 2021. In Silico ADME/T Properties of Quinine Derivatives Using SwissADME and pkCSM Webservers. International Journal of Tropical Disease and Health. 42 (11):1-12.

[20] Tripathi, P., Ghosh, S., Talapatra, S. N. 2019 Bioavailability Prediction of Phytochemicals Present in Calotropis procera (Aiton) R. Br. By Using SwissADME Tool. World Scientific News. 131:147-163.

[21] Bakchi, B., Krishna, A. D., Sreecharan, E., Ganesh, V. B. J., Niharika, M., Maharshi, S., Puttagunta, S. B., Sigalapalli, D. K., Bhandare, R. R. dan Shaik, A. B. 2022. An Overview on Applications of SwissADME Web Tool in The Design and Development of Anticancer, Antitubercular and Antimicrobial Agents: A Medicinal Chemist's Perspective. Journal of Molecular Structure. 1259:1-32.

[22] Riyadi, P. H., Susanto, E., Anggo, A. D. dan Arifin, M. H. 2023. In Silico Analysis of Small Molecules from Spirulina platensis as a Loop Dierutic and Anti-Inflammatory Agent. ARPN Journal of Engineering and Applied Sciences. 18 (13):1554-1566.

[23] Raschka, S., Wolf, A. J., Bemister-Buffington, J. dan Kuhn, L. A. 2018. Protein-Ligand Interfaces are Polarized: Discovery of a Strong Trend for Intermolecular Hydrogen Bonds to Favor Donors on The Protein Side with Implications for Predicting and Designing Ligand Complexes. Journal of Computer Aided Molecular Design. 32 (4):511-528.

[24] Lagorce, D., Douguet, D., Miteva, M. A. dan Villoutreix, B. O. 2017. Computational Analysis of Calculated Physicochemical and ADMET Properties of Protein-Protein Interaction Inhibitors. Scientific Reports. 7 (1):1-15.