ORIGINAL ARTICLE

Antihypertensive Activity of Peptides Derived from Toman Fish Albumin (*Channa micropeltes*): *In-silico* Angiotensin-Converting Enzyme Inhibitory Study

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ABSTRACT

The peptides from protein hydrolysis can be as bioactive peptides. Currently, the process of protein hydrolysis can be done *in-silico* technique, an alternative to bioactive peptide identification more effectively and efficiently. This study aims to predict bioactive peptides *in-silico* technique of albumin hydrolysis from Toman Fish (*Channa micropeltes*), which had the potential as an antihypertensive drug. Toman fish albumin sequence (A0A191TFW5) was obtained from the UniProt database. The identification of bioactive peptides was performed by simulating enzymatic hydrolysis with three human digestive enzymes: trypsin, chymotrypsin, and pepsin. The hydrolysis simulation of albumin was conducted using the ExPASy PeptideCutter program. The generated peptides' potential activities, solubility, and ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties were predicted using various online prediction tools. Molecular docking was performed on the bioactive peptides to determine the Gibbs free energy (ΔG) and to illustrate the interaction between the bioactive peptides and the active site of the Angiotensin Converting Enzyme (ACE) as a comparison was used captopril which was a commercial ACE inhibitor. The results showed that bioactive peptide candidates were AI, VL and LVP. These peptides were potentially a candidate for alternative antihypertensive drugs.

Keywords: Active peptides, Albumin, Channa micropeltes, ACE-inhibitor, Enzymatic hydrolysis, Antihypertension, Molecular docking

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INTRODUCTION

Toman fish (*Channa micropeltes*) is a genus of fish with snakehead Fish (*Channa striata*) that was widely spread in Indonesia and Asian. Toman fish has been associated with the health sector due to its high protein content and comprehensive amino acid profile. The sequences and pattern of amino acids have certain functional relationships, for example, as antihypertension.[1] Toman fish contains 22.1% protein, of which the dominant amino acids composition are Glu, Asp and Lys.[2]

The extract from Toman fish has been recognized for its ability to aid in the healing of diabetic wounds, provide an anti-liver damage effect and exhibit analgesic properties.[3-6] It is believed that peptides derived from the hydrolysis of Toman fish proteins possess health benefits similar to those found in bioactive peptides from snakehead fish.[7] Peptides from snakehead fish protein hydrolysates have been reported to exhibit activities such as ACE inhibition and antihypertensive effects.[8,9]

Angiotensin-converting enzyme (ACE) is a crucial enzyme in the regulation of blood pressure in humans. Various synthetic antihypertensive drugs have been commonly used as ACE inhibitors in the management of hypertension. However,

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the use of these synthetic antihypertensive agents has been associated with several risks, including side effects such as hypotension, liver damage, heart damage, diabetes, and kidney damage.[10] However, the researchers looked for alternative natural ACE inhibitors whose impacts were relatively small and not risky and do not cause dependence. One type of drugs that began to be studied was bioactive peptides as ACE inhibitors.[11]

Some of the natural ACE inhibitor agents that have been studied were sourced from food proteins.[12,13] Some fragments of peptides from milk have also been found to have antihypertensive properties.[14,15] Two ACE-inhibitory peptides, with the amino acid sequences VPAAPPK (IC-50 = 0.45 μM) and NGTWFEPP (IC-50 = 0.63 μM), have been identified.[7] The peptides LYPPP and YSMYPP sequences of snakehead fish have been identified.[8] The peptides Ile-Pro-Pro and Val-Pro-Pro exhibit ACE inhibitory activity.[16] ACE inhibitor peptides can be generated through the proteolytic cleavage by digestive enzymes in the stomach and small intestine.[17] Digestive enzymes that can hydrolyze proteins were pepsin, trypsin and chymotrypsin.[18]

In-silico studies to obtain bioactive peptides as several researchers have conducted ACE inhibitors. Bioactive peptides were found from the virtual results of screening cow's milk casein.[19] Peptides with EALPHVPIFDR sequences show strong binding affinity and high antihypertensive activity from molecular docking results. Aromatic amino acids, particularly tyrosine (Tyr), have been identified as crucial residues in the design of bioactive peptides and pharmaceuticals, including ACE inhibitors.[20] The tripeptide HGR (His-Gly-Arg), identified through virtual screening of the nebulin protein from *Larimichthys crocea*, demonstrates significant ACE inhibitory activity with an IC50 value of 106 ± 1.35 μM.[21] In an *in-silico* study of bioactive peptides from *Pistacia vera* L. hydrolysates, a novel ACE inhibitory peptide named ACKEP was identified, with an IC_{50} value of 126 μM.[22] Two novel noncompetitive ACE inhibitors, Thr-Thr-Trp (TTW) and Val-His-Trp (VHW), demonstrated the highest inhibitory activity, with IC_{50} values of 0.61 ± 0.12 μM and 0.91 ± 0.31 μM, respectively.[23]

This study aims to explore bioactive peptides from albumin Toman fish hydrolysates. The *in-silico* methods were carried out through virtual screening of peptide fragments from enzymatic hydrolysis. Three types of enzymes were used to hydrolyze, namely pepsin, trypsin and chymotrypsin. The proteolytic enzyme was found in the human digestive system. The results of enzymatic hydrolysis were to produce bioactive peptides that still have physiological activities, especially when applied *in vivo* methods.

MATERIALS AND METHODS

Protein selection

The albumin protein sequence of Toman fish (UniProt code A0A191TFW5) was retrieved from the UniProt database (https://www.uniprot.org/) and formatted in FASTA.[24] The protein structure of the angiotensin converting enzyme (PDB code 2X8Z), specifically the crystal structure of the AnCE-captopril complex, was obtained from the RCSB PDB database (https://www.rcsb.org/), and used as the target for molecular docking studies.[25]

Protein hydrolysis

Protein hydrolysis was conducted using the ExPASy PeptideCutter program (http://web.expasy.org/peptide_cutter/) available on the Expert Protein Analysis System (ExPASy) Molecular Biology Server.[26] Peptides with fewer than 20 amino acids were selected for further analysis.

Peptide screening

The antihypertensive properties of the hydrolyzed peptides were evaluated using the AHTpin tool (http://crdd.osdd.net/raghava/ahtpin/).[27] Toxicity parameters were assessed via the pkSCM website (http://biosig.unimelb.edu.au/pkcsm/prediction), while allergenicity was predicted using the AllerTOP website (https://www.ddgpharmfac.net/AllerTOP/feedback.py).[28] The IC50 values were determined using the tool available at http://crdd.osdd.net/servers/ic50avp. Additionally, the Toxinpred website (https://webs.iiitd.edu.in/raghava/toxinpred/index.html) was used to predict the toxic and non-toxic properties of the peptides.[29]

Molecular docking

The molecular docking procedure involved preparing the structures of the molecules in SMILES format. Peptide sequences were converted to SMILES format using the NovoPro website (https://www.novoprolabs.com/tools/convert-peptide-to-smiles-string). The hydrolyzed peptides, serving as ligands, and the ACE protein target were prepared and visualized using Chimera 1.14 (https://www.cgl.ucsf.edu/chimera/download.html).[30] Molecular docking was performed using the SWISS-DOCK program (http://www.swissdock.ch/docking).[31]

RESULTS AND DISCUSSIONS

Albumin hydrolysis

The albumin protein (COX1) from Toman fish (UniProt code A0A191TFW5) is a component of cytochrome C oxidase, which is the final enzyme in the mitochondrial electron transport chain responsible for driving oxidative phosphorylation. Cytochrome C oxidase facilitates the transfer of electrons derived from NADH and succinate to molecular oxygen, generating an electrochemical gradient across the inner mitochondrial membrane that drives transmembrane transport and ATP synthesis. This enzyme catalyzes the reduction of

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>tr|A0A191TFW5|A0A191TFW5_CHAMH Cytochrome c oxidase subunit 1
OS=Channa micropeltes OX=64149 GN=COX1 PE=3 SV=1
MAITRWLFSTNHKDIGTLYLIFGAWAGMVGTALSLLIRAELSQPGALLGDDQIYNVIVTA
HAFVMIFFMVMPMMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSAVEA
GAGTGWTVYPPLASNLAHAGASVDLTIFSLHLAGVSSILGAINFITTIINMKPPAISQYQ
TPLFVWAILITAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMISHIVAYYAGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGLDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGAIKWETPLLWALGFIFLFTIGGLTGIVLAN
SSLDIILHDTYYVVAHFHYVLSMGAVFAIMGAFIHWFPLFSGYTLHNTWTKIHFGVMFVG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNTISSIGSMISLVAVTMFLYIIWEAFVAKR
EVLSAELTTTNAEWLHGCPPPYHTFEEPAFVQVRSN
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Figure 1: COX1 Toman Fish Sequence (C. micropeltes) in FASTA format

oxygen to water and is a crucial part of the respiratory chain. The COX1 protein sequence from Toman fish, consisting of 516 amino acids, is depicted in Figure 1. This protein was released in April 2016.[24]

In-silico digestion was performed using the ExPASy PeptideCutter program (http://web.expasy.org/peptide_cutter/). Hydrolysis of the COX1 protein with three digestive enzymes pepsin, trypsin, and chymotrypsin, yielded 23 dipeptides, as detailed in Table 1. Additionally, 20 tripeptides were produced from the hydrolysis of COX1 protein, as shown in Table 2. Small peptides, such as dipeptides and tripeptides, generally exhibit minimal toxic properties.[32] Furthermore, existing research indicates that shorter peptide chains more readily interact with the active site of ACE, owing to their lower molecular weight and improved absorption.[33] Thus, dipeptides and tripeptides were selected as potential candidates for antihypertensive drug development.[34]

Virtual screening

Table 1 shows the detailed results of the hydrolysis, including the cut positions of the sequences, peptide sequences, mass peptides and pIC-50. The pIC-50 value was used as an initial screening. The peptides whose pIC-50 was greater than 4.5 have the potential as antihypertensive. The peptides whose pIC-50 is less than 4.5 do not have antihypertensive properties. IC-50 values are converted to the pIC-50 scale, where higher pIC-50 values represent exponentially greater inhibitory potency. The pIC-50 is typically expressed in terms of molar concentration (mol/L, or M).[35] The evaluated the value of pIC-50, there were 4 dipeptides that have the potential as antihypertensive, namely AI, VL, PR and HY.

Table 2 shows that the evaluated pIC50 > 4.5, that indicate as potential as a candidate for antihypertensive drugs. There were 11 tripeptides that have the potential to be

Table 1: Dipeptide hydrolysis results

Num of Cleveage	*Peptide sequence*	*Mass peptide*	*PIC-50*
24	GA	146.146	2.70
402	SG	162.145	2.07
432	LG	188.227	2.06
80	GN	189.171	2.98
326	AT	190.199	3.60
188	AI	202.253	5.47
475	EA	218.21	2.00
199	SL	218.253	3.64
287	IV	230.307	3.84
381	VL	230.307	4.89
339	LL	244.334	3.60
472	II	244.334	3.87
411	TK	247.294	3.59
504	HT	256.261	3.59
398	FP	262.309	3.50
395	IH	268.316	3.74
96	PR	271.319	5.39
8	LF	278.351	3.71
184	LF	278.351	3.71
400	LF	278.351	3.71
233	QH	283.287	3.61
38	IR	287.362	3.82
379	HY	318.332	4.58

Table 2: Tripeptide hydrolysis results

Num of Cleveage	*Peptide sequence*	*Mass peptide*	*PIC50*
264	AGK	274.320	3.93
296	TVG	275.305	4.29
446	PDA	301.299	4.77
486	SAE	305.288	4.39
417	GVM	305.392	4.49
479	VAK	316.401	4.37
251	PGF	319.360	4.38
84	LVP	327.424	5.00
202	PVL	327.424	5.20
41	AEL	331.369	4.24
182	QTP	344.368	4.83
337	ETP	345.353	4.92
366	DII	359.423	4.89
483	EVL	359.423	4.75
66	VMI	361.500	5.10
429	PQH	380.404	3.82
443	SDY	383.358	5.22
213	TDR	390.396	4.90
268	EPF	391.424	4.97
414	IHF	415.492	3.71

Table 3: Prediction of peptides properties

Peptide sequences	*SVM score*	*Toxic predictions*	*Mass*
AI	-0.8	Non-Toxin	202.27
VL	-0.8	Non-Toxin	230.33
PR	-0.8	Non-Toxin	271.33
HY	-0.8	Non-Toxin	318.35
PDA	-0.78	Non-Toxin	301.32
LVP	-0.8	Non-Toxin	327.46
PVL	-0.83	Non-Toxin	327.46
QTP	-0.82	Non-Toxin	344.4
ETP	-0.83	Non-Toxin	345.38
DII	-0.78	Non-Toxin	359.46
EVL	-0.79	Non-Toxin	359.46
VMI	-0.78	Non-Toxin	361.54
SDY	-0.82	Non-Toxin	383.38
TDR	-0.83	Non-Toxin	390.42
EPF	-0.83	Non-Toxin	391.45

Table 4: Docking Results

Ligan interaction	*Delta G (kcal/mol)*	*H Bond*	*Hydrophobic*
Al	-10.21	His-337; Glu-368; Lys-495	Gln-265; Ala-338; His-367; His-497; Tyr-507
VL	-10.35	Glu-368; Lys-495	Gln-265; His-337; Ala-338; Thr-364; His-367; Phe-441; His-497; Tyr-507
PDA	-8.78	Arg-86 (2 bonds), Leu-194	Lys-98, Thr-101, Asn-196,
LVP	-10.13	Gln-265, Lys-495	His-337, Ala-338, Thr-364, His-367, His-371, Glu-368, Gill-395, Tyr-507
ETP	-8.74	Lys-38, Gln-379	Ala-37, Gln-381, Arg-386, Leu-345, Thr-346, Phe-383
VMI	-9.26	Lys-98, Asp-206	Phe-197, Glu-214
EPF	-9.79	Lys-519 (2 bonds),	Glu-574; Glu-584, Ser-520, Lys-580 (3 bonds)
Captopril (Redocking)	-7.84	Arg-427 (2 bonds)	Tyr-528; Glu-584; Pro-588
Captopril (native)	--	Gln-265; His-337; His-367; His-371; Glu-395; Lys-495; Tyr-504; Tyr-507	Ala-338; Glu-368; Phe-441

antihypertensive, namely: PDA, LVP, PVL, QTP, ETP, DII, EVL, VMI, SDY, TDR, EPF.

Dipeptides and tripeptides were screened with toxicity, solubility, bioactivity and ADME properties to determine its potential as an antihypertensive drug. The virtual screening results are shown in Table 3. If the SVM (support vector machine) score was less than zero, then all dipeptides and tripeptides were potential as candidates for antihypertensive drugs. There were 15 peptides that have the potential to be antihypertensive drugs, consisting of 4 dipeptides and 11 tripeptides.

The virtual screening process was continued against 15 dipeptides and tripeptides as candidates for antihypertensive drugs in terms of allergenity and toxicity properties with different servers. The results of the virtual screening were contained with molecular docking in Table 4. Peptides that have the potential as antihypertensive drugs were 7 peptides, with details of 2 dipeptides, namely AI, VL and 5 tripeptides, namely PDA, LVP, ETP, VMI and EPF. Furthermore, the peptides will be docked with ACE target enzyme (PDB ID 2X8Z). Molecular docking was performed to see amino acid residues bound with ACE, that bind to peptides ligands. ACE inhibitor native ligand was captopril which was widely used as an antihypertensive drug.

In addition to evaluating toxicity, allergenicity, and non-allergenicity, drugs must also adhere to Lipinski's five rules. Compounds that do not comply with these rules typically exhibit poor pharmacokinetic properties, including inadequate absorption, rapid metabolism and excretion, poor distribution, and potential toxicity. Lipinski's five rules provide a useful framework for predicting the drug-likeness of compounds, filtering out those with molecular weights greater than 500, log P values exceeding 5, more than 5 hydrogen bond donors, and more than 10 hydrogen bond acceptors.[36] Table 4 indicates that 9 peptides were identified as non-toxic and non-allergenic, and 7 peptides met Lipinski's criteria.

Molecular docking

Molecular docking between ACE and the dipeptides and tripeptides (i.e., AI, VL, PDA, LVP, ETP, VMI, and EPF) was conducted to identify tightly bound peptides using the SwissDock website. The molecular interactions were explored with the Chimera 1.14 program, a versatile visualization tool.

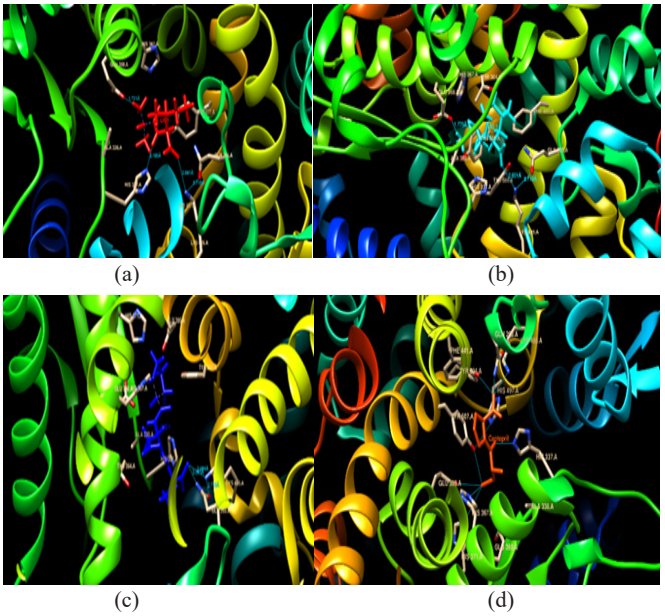

Figure 2: Docking results of peptides against ACE (PDB ID 2X8Z). The atoms of the ligands are colored according to their type. The figure displays the amino acid residues and hydrogen bond networks within the binding pocket. (a) Binding mode of the peptide AI with ACE. (b) Binding mode of the peptide VL with ACE. (c) Binding mode of the peptide LVP with ACE. (d) Binding mode of captopril with ACE

Table 4 and Figure 2 demonstrate that the active site of ACE interacting with the native captopril includes residues Gln-265, His-337, His-367, His-371, Glu-395, Lys-495, Tyr-504, and Tyr-507, which form hydrogen bonds, while Ala-338, Glu-368, and Phe-441 participate in hydrophobic interactions. The dipeptide AI binds to ACE through hydrogen bonds with His-337, Glu-368, and Lys-495, and hydrophobic interactions with Gln-265, Ala-338, His-367, His-497, and Tyr-507. Similarly, the dipeptide VL binds through hydrogen bonds with Glu-368 and Lys-495, and hydrophobic interactions with Gln-265, His-337, Ala-338, Thr-364, His-367, Phe-441, His-497, and Tyr-507. Both

dipeptides, AI and VL, exhibit strong binding to ACE with ΔG values of -10.21 kcal/mol and -10.35 kcal/mol, respectively. Figure 2 illustrates that the AI–three hydrogen bonds stabilize ACE complex, while the VL–ACE complex is stabilized by two hydrogen bonds. The dipeptides AI and VL are identified as potential strong candidates for antihypertensive drugs. Additionally, it has been reported that the dipeptide FP forms hydrophobic interactions with residues Ala-354, Ala-356, Phe-391, Phe-512, and Val-518, as well as with His-353, His-383, His-387, His-410, His-513, Glu-384, Glu-411, and Arg-522.[37]

Figure 2 illustrates that the tripeptide LVP binds to ACE via hydrogen bonds with residues Gln-265 and Lys-495, and through hydrophobic interactions with residues His-337, Ala-338, Thr-364, His-367, His-371, Glu-368, Glu-395, and Tyr-507. The molecular interaction of the tripeptide IPA with ACE involves hydrophobic interactions with Ala-354, Ala-356, Phe-391, Phe-512, and Val-518, as well as hydrophilic interactions with His-353, His-383, His-387, His-410, His-513, Glu-384, Phe-411, and Arg-522. The aromatic residue Tyr-523 also contributes to the stabilization of these interactions.[37] The tripeptide LVP binds very strongly to ACE, with a ΔG of -10.13 kcal/mol, and Figure 2 indicates that the LVP–two hydrogen bonds stabilize ACE complex. Thus, LVP shows significant potential as an antihypertensive drug candidate. It has been reported that the tripeptide HGR (His-Gly-Arg) demonstrated effective ACE inhibitory activity, with the HGR–ACE complex stabilized by 14 hydrogen bonds with key ACE residues, including His-353, Glu-384, Ala-354, His-513, Tyr-523, and Lys-511.[21] Additionally, hydrogen bonding and hydrophobic interactions are crucial features for the efficacy of ACE inhibitory tripeptides.

An analysis of the amino acids interacting with peptide ligands identified three peptides AI, VL, and LVP as promising candidates for antihypertensive drugs due to their similarity to captopril. Understanding the interactions between ACE and these peptides is critical for identifying effective ACE inhibitors.[23,38] The interaction of captopril (used as a redocking ligand) with ACE was evaluated, revealing a ΔG value of -7.84 kcal/mol. Figure 2(d) demonstrates that captopril (native ligand) interacts with ACE at residues Gln-265, His-337, His-367, His-371, Glu-395, Lys-495, Tyr-504, and Tyr-507, highlighting these residues as crucial for ACE binding. The real interactions between ACE and the drugs are essential to screen potent ACE inhibitory peptides.[39]

CONCLUSION

The peptides AI, VL and LVP, the hydrolysis results in protein COX1 Toman fish with digestive enzymes, namely pepsin, trypsin and chymotrypsin, potentially as candidates for antihypertensive drugs as ACE inhibitors. AI, VL and LVP peptides were capable of binding with hydrogen bonds and hydrophobic interaction mostly ACE residues on the active side.

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