In Silico Evaluation of Soybean Protein as Bioactive Peptide for Anti-Thrombotic Agent by Molecular Docking Study

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ABSTRACT
Cardiovascular Diseases (CVDs) are a growing global concern, including in Indonesia. By 2021, strokes were the leading cause of cardiovascular diseases (CVD), affecting 87% of the global population, while in 2018, 33.3% of Indonesians experienced CVD primarily due to strokes. Thrombolysis mediated by the α-thrombin (IIa) receptor and its interaction with coagulation factors in cardiac blood vessels underlies stroke development. Although chemical drugs like heparin are available, their potential side effects and adverse reactions are a concern. This study investigates the activity and interaction of anti-thrombotic bioactive peptides derived from soybean protein as potential thrombotic prevention agents, focusing on their role within food-derived bioactive peptides. Three peptides were identified, with the 7S globulin exhibiting the highest activity. Molecular docking analysis using PyMol, PyRx, and Discover Studio screened the peptides, showing no harmful peptides except for the β-conglycinin subunit variant that could potentially act as an allergen for individuals hypersensitive to β-conglycinin, as determined by ToxinPred and AlgPred analyses, while the DEE peptide demonstrated the highest binding affinity (-6.5 kcal/mol). This study highlights the potential of anti-thrombotic bioactive peptides from soybean protein as preventive agents against thrombosis, emphasizing the significance of the 7S globulin peptide and the superior binding affinity of the DEE peptide for potential therapeutic options in managing CVD-related complications.

Keywords: *anti-thrombotic peptide, food-derived bioactive peptide, molecular docking*

1. INTRODUCTION

Stroke is a disease of cardiovascular diseases (CVDs) with a high mortality rate on a global and Indonesian scale. Based on the Indonesian Ministry of Health's Basic Health Research in 2018, stroke cases had a percentage of 33.3% with 10.9% of its are the elderly (55-64 years). Stroke is caused by the blockage of the platelet in the blood vessels [1]. Thrombosis is the main cause of embolism, as this phenomenon results in the production of thrombin, which acts as a receptor for blood platelets to interact with each other. The interaction of α-thrombin (IIa) and thrombotic factor occurs precisely at the active site of the receptor at residues His57, Asp102/109, and Ser195 as well as in the exosite I or exosite II receptor [1]. This active site has fibrinogen which is convent soluble and can convert fibrinogen into fibrin filaments when interact with thrombotic factors. Furthermore, when factors V, XII, and XI are active, they will cause negative feedback and form a thrombus [2].

Anti-thrombotic is currently obtained from casein milk (animal) protein through the inhibition of α-thrombin (IIa) receptors [3]. Furthermore, other protein sources have a potential to be explored for anti-thrombotic agent development. Soybean protein has bioactive activity and can be an alternative as an anti-thrombotic peptide. Soybean protein is composed of two storage proteins, namely β-conglysinin (7S) and glycine (11S) subunits. The β-conglysinin subunit has three different types of chains namely β-chain, α-chain, and α'-chain. Glycine is composed of acidic (A) and basic (B) subunits [4]. The aim of this research is to conduct an in

J. Sukweenadhi and F. Setiawan (eds.), *Proceedings of the Conference on Natural Resources And Life Sciences 2022 (NRLS-BIO 2022)*, Advances in Biological Sciences Research 38,
https://doi.org/10.2991/978-94-6463-322-1_5

silico evaluation of the bioactive peptides activity from soybean protein and predict its toxicity and allergenic properties. Furthermore, this molecular study examines the amino acid residues of anti-thrombotic peptides that interact with α-thrombin (IIa) as a ligand. The findings of this research may serve as a guide or supporting analysis for the *in vitro* or *in vivo* validation stage.

2. MATERIAL AND METHOD

The proteins of interest were retrieved from the Uniprot platform, and their codes are listed in **Table 1**. The anti-thrombotic peptide sequences were predicted by utilizing the BIOPEP platform and constructed using PyMOL 2.5. The bioactive scores of the anti-thrombotic peptides will be calculated to evaluate their characteristics in response to anti-thrombotic biological activity with the Peptide Ranker server. Peptides with a probability rank of above 0.5 and close to 1.0 are considered to have bioactive activity. Allergies are analyzed using AlgPred, which takes amino acids and their sequences, and IgE epitopes as input. The ToxinPred server is utilized to evaluate the toxicity levels of the peptides. Peptides are considered toxic if their threshold is >0.0. Molecular interactions between each peptide and the α-thrombin (PDB: 2BVS) receptor are analyzed using PyRx to determine the lowest binding affinity energy, and a control complex is used for comparison. The docking results are visualized using the BIOVIA Discovery Studio [5].

Table 1 Soybean Protein Evaluation

Constituent Soybean Protein	Existing Anti-Thrombotic Peptide	Allergen prediction
7S globulin (Uniprot: P13917)	✓	Non
11S glycin (Uniprot: P04347)	✓	Non
β-conglycinin, β-chain (Uniprot: P25974)	✓	Allergen
β-conglycinin, α-chain (Uniprot: A0A445F555)	✓	Allergen
β-conglycinin, α'-chain (Uniprot: P11827)	✓	Allergen

3. RESULT AND DISCUSSION

3.1 Anti-thrombotic peptide activity

Table 1 showed that all soybean protein constituents possessed bioactive peptides that exhibited anti-thrombotic activity. The bioactive anti-thrombotic peptide fragment that occurs in the three analysis sources can be seen in **Figure 1** with the 7S globulin showing the highest bioactive frequency value of 0.098 A but still lower bioactive frequency compared with chemical synthesis as anti-thrombotic activity [2]. There were 3 anti-thrombotic peptides found that have 2 peptides such as GP = 0.90 and PG = 0.87 above the threshold (≥ 0.5) as shown in **Table 2** and one peptide which is DEE = 0.03 below the threshold. These peptides (GP, PG, and DEE) are suspected to have better bioactivity, where a value close to 1.0 is the best peptide to be a bioactive agent [6]. These peptides will be used as further references for molecular docking analysis.

3.2 Toxicity and Allergenic Properties Prediction of Anti-thrombotic Candidate

The sequences recognized by the IgE epitope on the constituent proteins:

- **β-conglysinin, β-*chain***

MMRVRFPLLVLLGTVFLASVCVSLKVREDENNPF
YLRSSNSFQTLFENQNGRIRLLQRFNKRSPQLENL
RDYRIVQFQSKPNTILLPHHADADFLLFVLSGRAI
LTLVNNDDRDSYNLHPGDAQRIPAGTTYYLVNPH
DHQNLKIIKLAIPVNKPGRYDDFFLSSTQAQQ**SYL**
QGFSHNILETSFHSEFEEINRVLFGEEEEQRQQEG
VIVELSKEQIRQLSRRAKSSSRKTISSEDEPFNLRSR
NPIYS**NNFGKFFEIT**PEKNPQLRDLDIFLSSVDINE
GALLLPHFNSKAIVILVINEGDANIELVGIKEQQQK
QKQEEEPLEVQRYRAELSEDDVFVIPAAYPFVVN
ATSNLNFLAFGINAENN**QRNFLAGEKD**NVVRQIE
RQVQELAFPGSAQDVERLLKKQRESYFVDAQPQ
QKEEGSKGRKGPFPSILGALY

- **β-conglysinin, α-*chain***

MMRARFPLLLLGLVFLASVSVSFGIAYWEKENP
KHNKCLQSCNSERDSYRNQACHARCNLLKVEKE
ECEEGEIPRPRPRPQHPEREPQQPGEKEEDEDEQP
RPIPFPRPQPRQEEEHEQREEQEWPRKEEKRGEK
GSEEEDEDEDEEQDERQFPFPRPPHQKEERKQEE
DEDEEQQQESEESEDSELRRHKNKNPFLFGSNRF
ETLFKNQYGRIRVLQRFNQRSPQLQNLRDYRILE
FNSKPNTLLLPNHADADYLIVILNGTAILSLVNND
DRDSYRLQSGDALRVPSGTTYYVVNPDNNENLR
LITLAIPVNKPGRFESFFLSSTEAQQ**SYLQGFSRNI**
LEASYDTKFEEINKVLFSREEGQQQGEQRLQESVI
VEISKEQIRALSKRAKSSSRKTISSEDKPFNLRSRD
PIYSNKLGKFFEITPEKNPQLRDLDIFLSIVDMNEG
ALLLPHFNSKAIVILVINEGDANIELVGLKEQQQE
QQQEEQPLEVRKYRAELSEQDIFVIPAGYPVVVN
ATSNLNFFAIGINAENNQRNFLAGSQDNVISQIPS
QVQELAFPGSAQAVEKLLKNQRESYFVDAQPKK
KEEGNKGRKGPLSSILRAFY

- **β-conglysinin, α'-*chain***

MMRARFPLLLLGVVFLASVSVSFGIAYWEKQNPS
HNKCLRSCNSEKDSYRNQACHARCNLLKVEEEEE
CEEGQIPRPRPQHPERERQQHGEKEEDEGEQPRPF
PFPRPRQPHQEEEHEQKEEHEWHRKEEKHGGKGS
EEEQDEREHPRPHQPHQKEEEKHEWQHKQEKHQ
GKESEEEEEDQDEDEEQDKESQESEGSESQREPRR

HKNKNPFHFNSKRFQTLFKNQYGHVRVLQRFNK
RSQQLQNLRDYRILEFNSKPNTLLLPHHADADYLI
VILNGTAILTLVNNDDRDSYNLQSGDALRVPAGT
TYYVVNPDNDENLRMITLAIPVNKPGRFESFFLSS
TQAQQ**SYLQGFSKNI**LEASYDTKFEEINKVLFGR
EEGQQQGEERLQESVIVEISKKQIRELSKHAKSSSR
KTISSEDKPFNLRSRDPIYSNKLGKLFEITPEKNPQ
LRDLDVFLSVVDMNEGALFLPHFNSKAIVVLVIN
EGEANIELVGIKEQQQRQQQEEQPLEVRKYRAEL
SEQDIFVIPAGYPVVVNATSDLNFFAFGINAENN**Q**
RNFLAGSKDNVISQIPSQVQELAFPGSAKDIENLI
KSQSESYFVDAQPQQKEEGNKGRKGPLSSILRAF
Y

Note: The sequences highlighted in grey are anti-thrombotic peptides candidate, while the sequences highlighted in **bold** are sequences recognized by the IgE epitope.

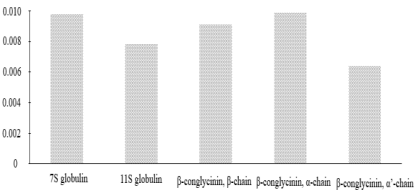

Figure 1 Graph of the anti-thrombotic bioactive peptide value (A).

Allergy analysis with AlgPred in **Table 1** and the amino acid sequence illustrated that there were potential allergies in soybeans which are β-conglycinin, β-*chain*; β-conglycinin, and α-*chain*; β-conglycinin. Based on **Table 2**, no potential toxic peptide was found. This could be seen from the SVM value which was negative or did not exceed the limit (threshold score > 0.0). Therefore, the three peptides─GP, PG, and DEE─ that are predicted to be anti-thrombotic are safe to be used as therapeutic agents for drug development and food-derived bioactive peptides [7].

3.3 MOLECULAR DOCKING STUDY

Table 2 was used to analyze the results of the molecular docking. The control compounds and three peptides (GP, PG, and DEE) were placed at specific locations and compared to the control compounds. Among the peptides, DEE was found to bind to both catalytic sites of α-thrombin, specifically at the active site gap and pocket S1α-thrombin. The binding involves hydrogen interaction with Cys191, Gly193, Glu192, and Ser195, as well as hydrophobic binding to His57 and Trp60A. The binding affinity of the GPR ligand was -6.5 kcal/mol, which is the closest to the binding energy of control 2CE, which was -6.9 kcal/mol. DEE ligand is not recommended as an anti-thrombotic medicine alternative because DEE peptide has the lowest bioavailability based on PeptideRanker.

4. CONCLUSION

Soybean protein, specifically the 7S globulin, has been found to offer health benefits as an anti-thrombotic agent. This constituent protein is non-toxic, but the β-conglycinin subunit can trigger hypersensitive reactions in the immune system by binding to the IgE epitope.

Based on molecular docking results, it was observed that anti-thrombotic peptides can interact positively with the inhibition of DEE peptides action towards α-thrombin, with a binding affinity of -6.5 kcal/mol. However, it is essential to conduct further studies on other protein sources to compare and provide more comprehensive anti-thrombotic drug development studies.

AUTHORS' CONTRIBUTIONS

F.S: Data curation, Writing original draft preparation, Reviewing, Editing. **M.I.F**: Conceptualization, Methodology, Visualization, Software. **A.K**: data interpretation, reviewing. **D.W**: reviewing, editing.

Table 2 Anti-thrombotic Peptide and Molecular Docking Evaluation

Ligand	PeptideRanker Score	Toxicity Prediction	SVM Score	Binding Affinity	Interaction bond	
					Hydrogen	Hydrophobic
GP	0.905487	Non	-0.79	-4.7	Trp215 Ser195 Gly219	Cys220
PG	0.877086	Non	-0.80	-5.1	Gly219 Ser195 Gly216 Asp189 Ala190 Ser214	Ala190
DEE	0.0306847	Non	-0.82	-6.5	Cys191 Gly193 Ser195 Glu192	His57 Tyr60A

Ligand	PeptideRanker Score	Toxicity Prediction	SVM Score	Binding Affinity	Interaction bond	
					Hydrogen	Hydrophobic
Control (2CE)	-	-	-	-6.9	Gly216 Gly219 Glu192	Trp60D Cyc191 Trp215 Ala190 Val213 Tyr228

ACKNOWLEDGMENTS

The authors would like to sincerely thank Mochamad Nurcholis, STP, MP, Ph.D., for his continuous encouragement and unwavering support during the course of this research. We deeply appreciate his valuable guidance and feedback which have significantly influenced the direction, quality, and academic growth of our study.

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