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Original Article

Unraveling the power of peptides from *Cucumaria frondosa* coelomic fluid as multitarget therapy of diabetic kidney disease: An in-silico study

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Abstract

Diabetic kidney disease is a condition characterized by persistent albuminuria, diabetic glomerular lesions, and a reduced glomerular filtration rate in people with diabetes. Peptides in *Cucumaria frondosa* coelomic fluid have been proven to provide antidiabetic and anti-inflammatory activity that can be used as one of the innovations in developing a multitarget therapy, especially in diabetic kidney disease. Therefore, the aim of this study was to unravel the power of peptide-based metabolites from *C. frondosa* coelomic fluid as multitarget therapy for diabetic kidney disease using an in-silico study. UCSF Chimera software was utilized to construct the three-dimensional structure of coelomic fluid peptides from *C. frondosa*. The toxicity and allergenicity of peptides were examined using the ToxinPred and AllerTop websites, respectively. From the PDBJ database, the 3D structures of protein kinase B, alpha isoform (AKT1); vascular endothelial growth factor receptor 2 (VEGFR2); epidermal growth factor receptor (EGFR); α-glucosidase; and glucokinase were obtained. Molecular docking was carried out using MOE Software. In this in-silico study, peptide 9 (-10.32 kcal/mol), peptide 1 (-9.41 kcal/mol), and peptide 3 (-9.55 kcal/mol) were shown to act as specific adenosine triphosphate-competitive inhibitors of EGFR, AKT1, and VEGFR2, respectively. Peptide 8 (-11.06 kcal/mol) can specifically inhibit α-glucosidase by binding to its active site. Peptide 1 (-9.80 kcal/mol) is predicted to specifically inhibit glucokinase activity by blocking its active side. Molecular dynamics simulations confirmed stable interactions with receptor proteins. In conclusion, *C. frondosa* coelomic fluid peptides have been shown not only to alleviate diabetic kidney disease but also to stabilize blood glucose levels and prevent hyperglycemia based on in-silico analysis.

Keywords: *Cucumaria frondosa*, diabetic kidney disease, in-silico, multitarget therapy, peptide-based therapy

Introduction

Diabetes mellitus is one of the most prevalent metabolic diseases caused by a combination of impaired ability to secrete insulin and the tissues' inability to react to insulin [1]. According to

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data from the International Diabetes Federation, the number of adults worldwide with a history of diabetes mellitus is expected to increase from 415 million in 2015 to 642 million in 2040, with an increase in prevalence from 8.8% to 10.4% [2]. The high number of diabetes cases in the world not only has an impact on public health but also has an impact on the global economic burden. Notably, the absolute global economic burden is expected to rise to $2.2 trillion by 2030 in the baseline [3].

Diabetes mellitus is known to cause complications in several organs, such as the heart (cardiopathy), eyes (retinopathy), nerves (neuropathy), kidneys (nephropathy), and others. One of the most common forms of diabetes complications is diabetic nephropathy. Diabetic nephropathy, also known as diabetic kidney disease (DKD), is a condition characterized by persistent albuminuria, diabetic glomerular lesions, and reduced glomerular filtration rate (GFR) in people with diabetes [4]. DKD is expected to improve significantly by starting, optimizing, and maintaining evidence-based pharmacological therapy with a therapeutic combination of RAS inhibitor + SGLT2i/GLP-1 RA + nonsteroidal MRA + statin [5]. However, exploratory studies are needed to identify drug candidates with multitarget activity to make therapy more effective and efficient.

The discovery of bioactive peptides from marine species has received much attention in recent years. Several investigations have been carried out to explore the potential of diverse marine creatures as abundant sources of essential amino acids and bioactive peptides. Among these, sea cucumber (*Cucumaria frondosa*) is known to be rich in benefits such as playing a role in anti-inflammatory, antioxidant, anticancer, anticoagulant, antifungal, and antidiabetic activities [6]. Looking at antidiabetic activity, sea cucumber coelomic fluid has been proven to inhibit proteins related to the pathogenesis of diabetes mellitus and DKD, including AKT, GLUT2/GLUT4, EGFR, and others [7]. The content of active compounds and peptides in sea cucumber coelomic fluid are proven to provide these biological effects so that further processing in the form of recombinant protein can be used as one of the innovations in developing a form of multitarget therapy, especially in DKD.

The potential of sea cucumber (*C. frondosa*) coelomic fluid peptides can be analyzed using in-silico analysis. This in-silico-based research will be the initial basis for identifying potential peptides that provide the most effective therapeutic inhibitory effects in DKD, stabilize blood glucose levels, and prevent hyperglycemia based on binding affinity. Therefore, in this study, research will be carried out to unravel the power of peptide-based metabolites from *C. frondosa* coelomic fluid as multitarget therapy for diabetic kidney disease using an in-silico study.

Methods

Ligand and protein preparation

The sea cucumber (*C. frondosa*) peptide sequence was obtained from the peptidomic profiling process using Liquid Chromatography-Mass Spectrometry (LC-MS) in the research of Zhang *et al.*, 2020 (**Table 1**) [8]. After obtaining the peptide sequence, a three-dimensional structure visualization was done using the UCSF Chimera application. The three-dimensional structure of the natural ligand in the form of adenosine triphosphate (ATP) (CID: 5957) was downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The three-dimensional structure of the control ligand for each target protein was isolated using the MOE v2022 application.

Target proteins were selected by analyzing various pathways involved in diabetes nephropathy/DKD pathogenesis. The selection of target proteins is also based on the results of the pharmacology network construction from previous research, which found 17 core target proteins in diabetes nephropathy/DKD [9]. Proteins that were identified included protein kinase B, alpha isoform (AKT1, PDB: 3MVH); vascular endothelial growth factor receptor 2 (VEGFR2, PDB: 4ASE); and epidermal growth factor receptor (EGFR, PDB: 1XKK). Furthermore, to stabilize blood glucose levels and prevent hyperglycemia, α-glucosidase (PDB: 5NN8) and glucokinase (PDB:1V4S) were chosen as target proteins. The protein structures were downloaded through the RCSB PDB website (https://www.rcsb.org/). All ligands and target proteins were prepared using the MOE v2022 application.

Toxicity and allergenicity prediction test

The toxicity test of *C. frondosa* peptides was carried out using the ToxinPred website (http://crdd.osdd.net/raghava/toxinpred/). The results of the toxicity analysis categorized the peptide as either toxic or non-toxic. The allergenicity test was conducted using the AllerTop website (https://www.ddg-pharmfac.net/AllerTOP/). The results of the allergenicity analysis categorized the peptide as either allergen or non-allergen.

Molecular docking

The MOE application was utilized to perform molecular docking. Using the SiteFinder feature, each target protein's ATP binding pocket or active site was used to guide the docking test specifically (**Table 1**). The "Ligand Interaction" feature visualized the molecular docking results from the previous step in two and three dimensions. The molecular docking results include binding affinity (kcal/mol) and root-mean-square deviation (RMSD). The coelomic fluid of *C. frondosa* peptide is considered to have potential as a target protein inhibitor if the binding affinity value is more negative (stronger) than the native/control ligand and has an RMSD value <2Å [10]. The 2D visualization results were used to show the similarity of the interaction formed between the coelomic fluid peptides of *C. frondosa* and ATP at the amino acid residues of the target protein. This indicates that the peptide can specifically inhibit the target protein's activity by preventing the native/control ligand from interacting with the target protein.

Table 1. The binding site amino acid residues of EGFR, AKT1, VEGFR2, α-Glucosidase, and glucokinase protein.

Protein	PDB ID	The binding site of target proteins
EGFR	1XKK	Lys716 Val717 Leu718 Gly719 Ser720 Gly721 Val726 Lys728 Ala743 Ile744 Lys745 Thr790 Gln791 Leu792 Me1793 Phe795 Gly796 Cys797 Asp800 Tyr801 Glu804 Arg841 Asn842 Leu844 Thr854 Asp855 Phe997 Tyr998 Leu1001 Met1002
AKT1	3MVH	Leu156 Gly157 Lys158 Gly159 Phe161 Gly162 Lys163 Val 164 Ala177 Lys179 Leu181 Ile186 Glu191 His194 Thr195 Glu198 Thr211 Met227 Glu228 Tyr229 Ala230 Glu234 Tyr272 Asp274 Lys276 Glu278 Asn279 Met281 Thr291 Asp292 Phe293 Gly294 Leu295 Phe438 Phe442
VEGFR2	4ASE	Pro812 Asp814 Leu840 Val848 Ala866 Val867 Lys868 Ala881 Leu882 Ser884 Glu885 Ile888 Leu889 Ile892 Val898 Val899 Val914 Val916 Glu917 Phe918 Cys919 Lys920 Phe921 Gly922 Asn923 Leu1019 Cys1024 Ile1025 His1026 Arg1027 Leu1035 Ile1044 Cys1045 Asp1046 Phe1047 Gly1048 Leu1049
α-Glucosidase	5NN8	Gln17 His120 Phe121 Asn164 Gln165 Asn167 Val168 Ser170 Val171 Met172 Asp175 Leu176 Met178 Phe179 Phe186 Gly187 Gly190 Tyr191 Ala194 Phe225 Val227 Leu229 Ala246 Phe249 His250 Leu253 Phe254 Tyr309 Thr310 Arg312 Ile314 Leu324 Gly325 Ile326 Leu327 Gln328 Ile332 Phe334 Trp345 Glu373 Trp417 Glu424 Trp425 Asn426 Gln427 Phe433
Glucokinase	1V4S	Asp78 Leu79 Gly80 Gly81 Thr82 Asn83 Phe84 Arg85 Thr149 Ser151 Phe152 Pro153 Thr168 Lys169 Asn204 Asp205 Thr206 Thr209 Ile225 Gly227 Thr228 Gly229 Cys230 Asn231 Glu256 Trp257 Gly258 Gln287 Glu290 Asp409 Gly410 Ser411 Lys414 Leu415 Ser441 Glu442 Glu443 Gly444 Ser445 Gly446

AKT1: alpha isoform; EGFR: epidermal growth factor receptor; PDB ID: protein data bank identification; VEGFR2: vascular endothelial growth factor receptor 2

Molecular dynamic simulation

Molecular dynamics simulations were conducted using YASARA Dynamic v4.3.13. Each sample was entered into the application using the Options menu, selecting the Set Target and Macro & Movie menus. Additionally, molecular dynamics simulations were built in the variable section—which included a physiological pH of 7.4 and a temperature of 310 K—and were run using macro input. In the next stage, the macro md_run sets the running time to 50,000 ps (50 ns). The Root Mean Square Deviation (RMSD) analysis was produced using the md_analyze macro. The macro md_analyzeres was then used to carry out the analysis.

Results

Toxicity and allergenicity prediction test

The results of the toxicity prediction test for *C. frondosa* coelomic fluid peptides indicate that peptides 1, 3, 4, 5, 6, 8, 9, and 10 are non-toxic, while peptides 2 and 7 are shown to be toxic. According to the allergenicity prediction test results, peptides 1, 3, and 5 are probably non-allergens. Meanwhile, peptides 2, 4, 6, 7, 8, 9, and 10 are probably allergens. So, peptides 1,3 and 5 can be considered safe (non-allergen and non-toxic). The results of the peptide structure, allergenicity, and toxicity prediction test of *C. frondosa* coelomic fluid peptides are presented in **Table 2**.

Table 2. The toxicity and allergenicity prediction test for *Cucumaria frondosa* coelomic fluid peptide

Peptide code	Sequence	Toxicity	Allergenicity
Peptide 1	YDWRF	Non-toxic	Non- allergen
Peptide 2	WPPNYQW	Toxic	Allergen
Peptide 3	WNWKV	Non-toxic	Non- allergen
Peptide 4	WNWKL	Non-toxic	Allergen
Peptide 5	VELWR	Non-toxic	Non- allergen
Peptide 6	TEFHLL	Non-toxic	Allergen
Peptide 7	RMCCCSPLK	Toxic	Allergen
Peptide 8	MMSLHL	Non-toxic	Allergen
Peptide 9	KMLWK	Non-toxic	Allergen
Peptide 10	EMEWR	Non-toxic	Allergen

Molecular docking protein target of EGFR

All *C. frondosa* peptides have binding affinity values much stronger than ATP (natural ligand) and erlotinib (control ligand). However, only peptides 7 (-10.47 kcal/mol) and 9 (-10.32 kcal/mol) had RMSD values <2 Å (**Table 3**). Although it has a stronger interaction than the others, peptide 7 is allergenic and toxic, while peptide 9 is an allergen (**Table 2**). Peptide 9 was considered more suitable for use as an ATP competitive inhibitor of EGFR proteins.

Based on 2D interaction analysis, there were seven interactions formed between peptide 9 and EGFR: two acidic hydrophilic interactions (Asp800 and Asp855), two basic hydrophilic interactions (Lys745 and Arg841), two polar hydrophobic interactions (Cys797 and Gly796), and one greasy hydrophobic interaction (Leu718) (**Table 4**). In comparison, ATP and EGFR formed five interactions: one acidic hydrophilic interaction (Asp855), one basic hydrophilic interaction (Lys745), one polar hydrophilic interaction (Thr854), and two greasy hydrophobic interactions (Leu718 and Val726). More interactions were established between peptide 9 and EGFR than that between ATP and EGFR. Peptide 9 and ATP share three types of interactions and amino acid residue similarities at Asp855, Lys745, and Leu718 (**Table 4**). The 3D interaction analysis showed that peptide 9 can bind to EGFR at the same site as ATP (**Figure 1**). These results indicate that peptide 9 is expected to inhibit the ATP binding pocket of EGFR specifically.

Table 3. The summary of molecular docking results

Protein	Peptide code	Binding affinity (kcal/mol)	RMSD (Å)
GFR	ATP	-9.84	0.98
	Erlotinib	-7.71	1.96
	Peptide 1	-10.07	2.24
	Peptide 2	-11.27	3.32
	Peptide 3	-9.69	1.94
	Peptide 4	-9.34	1.64
	Peptide 5	-9.32	1.78
	Peptide 6	-8.54	1.37
	Peptide 7	-10.47	1.71
	Peptide 8	-9.39	2.85
	Peptide 9	-10.32	1.79
	Peptide 10	-9.67	1.80
AKT1	ATP	-8.03	0.93
	GDC0068	-9.15	1.68
	Peptide 1	-9.41	1.57

Protein	Peptide code	Binding affinity (kcal/mol)	RMSD (Å)
	Peptide 2	-10.23	1.69
	Peptide 3	-9.55	2.78
	Peptide 4	-9.74	1.61
	Peptide 5	-9.17	1.89
	Peptide 6	-8.80	1.60
	Peptide 7	-10.56	1.81
	Peptide 8	-9.11	1.74
	Peptide 9	-10.05	2.33
	Peptide 10	-9.89	1.67
VEGFR2	ATP	-7.73	1.75
	Benzimidazole urea inhibitor	-7.50	1.35
	Peptide 1	-10.17	2.31
	Peptide 2	-11.85	3.43
	Peptide 3	-9.55	1.94
	Peptide 4	-10.00	3.29
	Peptide 5	-8.21	1.73
	Peptide 6	-9.17	1.71
	Peptide 7	-12.01	3.55
	Peptide 8	-10.77	1.90
	Peptide 9	-10.00	1.77
	Peptide 10	-8.98	2.54
α-Glucosidase	Acarbose	-10.37	1.92
	Peptide 1	-9.47	1.99
	Peptide 2	-11.47	2.58
	Peptide 3	-9.73	1.89
	Peptide 4	-8.97	1.86
	Peptide 5	-9.83	1.76
	Peptide 6	-10.76	1.40
	Peptide 7	-10.59	1.89
	Peptide 8	-11.06	1.65
	Peptide 9	-9.92	1.99
	Peptide 10	-9.66	1.86
Glucokinase	Glucokinase inhibitor	-7.57	1.80
	Peptide 1	-9.80	1.51
	Peptide 2	-10.14	1.85
	Peptide 3	-8.47	1.64
	Peptide 4	-9.40	1.88
	Peptide 5	-9.12	1.94
	Peptide 6	-8.82	1.77
	Peptide 7	-10.14	2.44
	Peptide 8	-10.27	2.46
	Peptide 9	-9.76	2.05
	Peptide 10	-8.61	1.85

ATP: adenosine triphosphate; RMSD: root-mean-square deviation

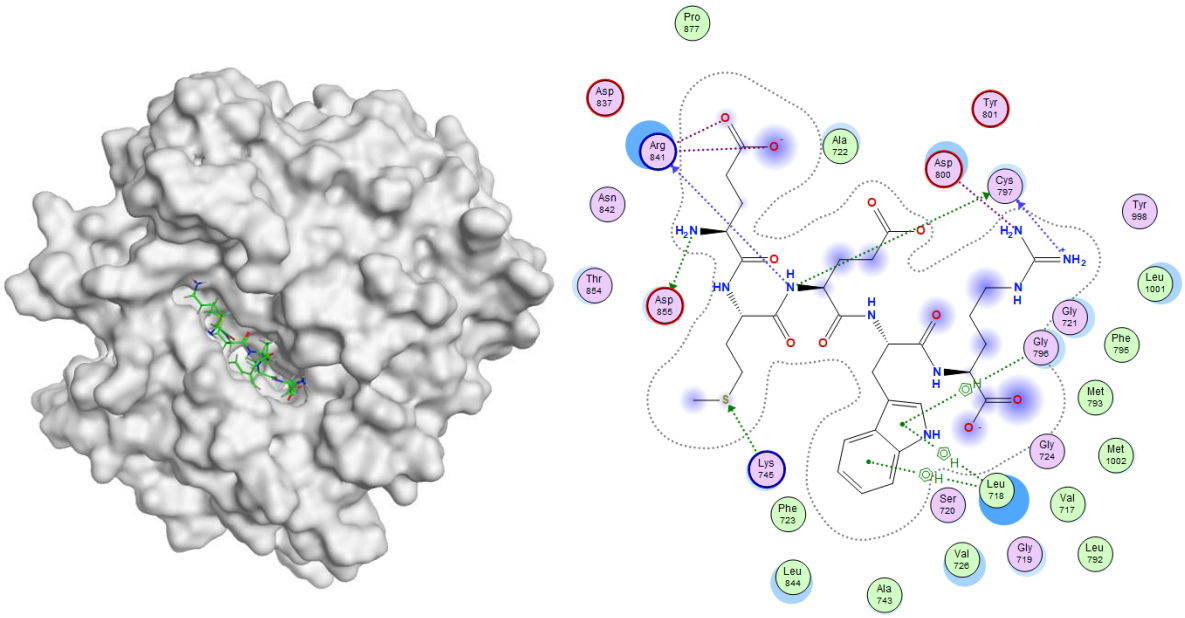

Figure 1. Two- and three-dimensional visualizations of EGFR complex with peptide 9.

Molecular docking protein target of AKT1

The molecular docking results between *C. frondosa* peptides and AKT1 protein show that all peptides from *C. frondosa* generally have binding affinity values stronger than ATP (-8.03 kcal/mol) and GDC0068 (-9.15 kcal/mol) (**Table 3**). However, peptides 3 and 9 have RMSD values of more than 2Å. Considering both toxicity and allergenicity, peptides 1 and 5 are particularly promising since they are non-toxic and non-allergenic (**Table 2**). Peptide 1 (-9.41 kcal/mol) is the most potential candidate from *C. frondosa* for ATP competitive inhibitors of AKT1 protein.

The interaction between peptide 1 and AKT1 resulted in six interactions: two acidic hydrophilic bonds (Glu228, Glu234), two basic hydrophilic bonds (Lys179, Lys276), and two hydrophobic bonds (Val164, Met281) (**Table 4**). In comparison, the interaction between ATP and AKT1 forms four interactions: three acidic hydrophilic (Glu228, Glu234, Asp292) and one greasy hydrophobic (Met227). Peptide 1 formed more hydrophobic bonds than ATP. Moreover, peptide 1 formed similar bonds in two residues that are the same as ATP, involving Glu228 and Glu234 (**Table 4**). The visualization of the AKT1 complex with peptide 1 in two- and three-dimensional formats is presented in **Figure 2**.

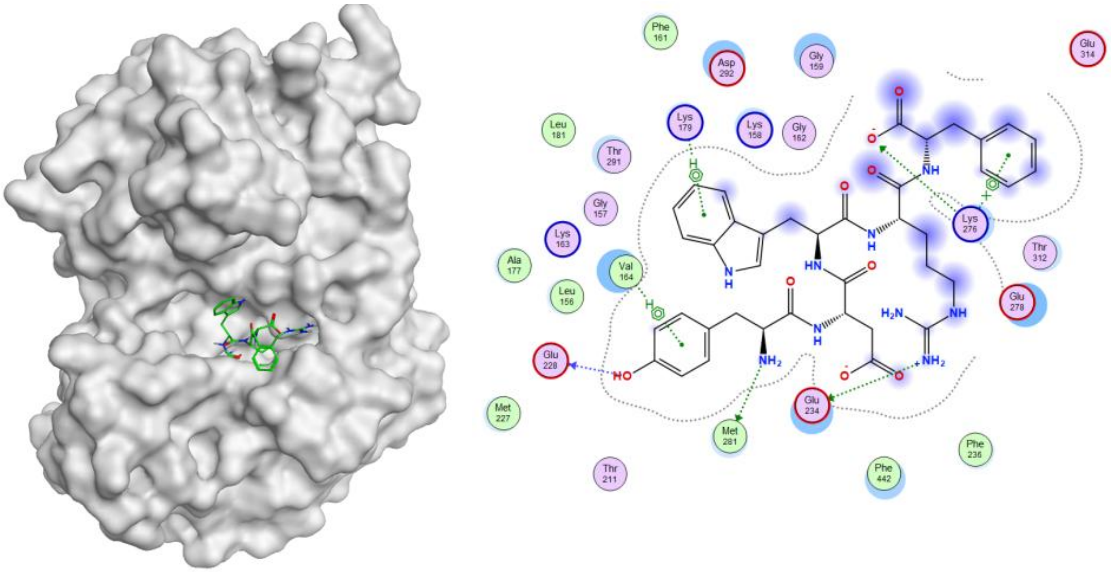

Figure 2. Two- and three-dimensional visualizations of AKT1 complex with peptide 1.

Molecular docking protein target of VEGFR2

The docking results between VEGFR2 and the peptides showed that peptides 3 (-9.55 kcal/mol), 5 (-8.21 kcal/mol), 6 (-9.17 kcal/mol), 8 (-10.77 kcal/mol), and 9 (-10.00 kcal/mol) binds to the ATP binding pocket with a lower binding affinity than ATP (-9.55 kcal/mol) and benzimidazole urea inhibitor (-7.50 kcal/mol). The molecular docking results have also been validated, as evidenced by the RMSD value <2 Å (**Table 3**).

Based on its toxicity and allergenicity properties, peptide 3 has the potential to be used as a candidate therapeutic agent because it is non-toxic and non-allergenic. The 2D interaction visualization analysis showed that peptide 3 formed more interactions than ATP (**Figure 3**). There are two similar amino acid residues, such as Asp814 (hydrophilic acidic) and His1026 (hydrophilic polar) (**Table 4**).

Molecular docking protein target of α-glucosidase

Docking between *C. frondosa* peptides and α-glucosidase proteins showed that peptides 6 (-10.76 kcal/mol), 7 (-10.59 kcal/mol), and 8 (-11.06 kcal/mol) had stronger binding affinity than Acarbose (-10.37 kcal/mol), the drug control. The RMSD values of these three peptides are also smaller than 2 Å (**Table 3**). Peptides 6 and 8 were found to be allergenic and non-toxic (**Table 2**).

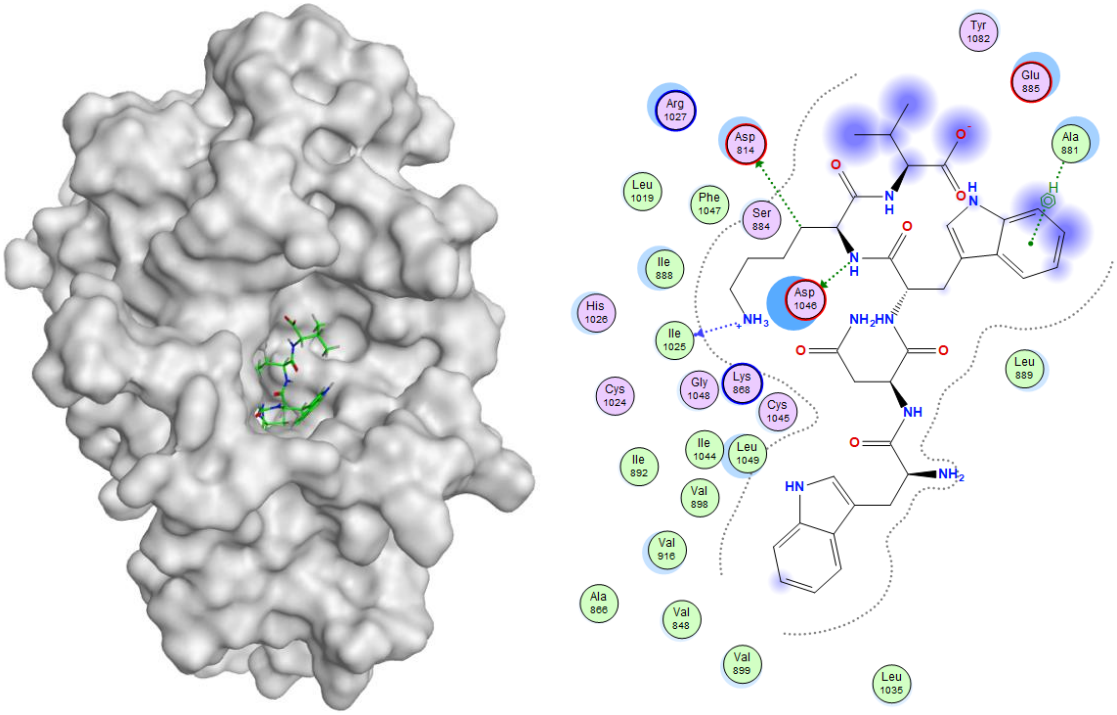

Figure 3. Two- and three-dimensional visualizations of VEGFR2 complex with peptide 3.

The analysis of 2D interaction visualization revealed that peptide 8 formed eight interactions (Glu373, Glu424, Arg312, Gln165, Asn164, His120, Gln17, and Trp425) with the α-glucosidase active sites containing two acidic hydrophilic interactions, one basic hydrophilic interaction, four polar hydrophilic interactions, and one greasy hydrophobic interaction (**Table 4**). Meanwhile, acarbose formed four interactions (His250, Ile326, Phe334, and Trp425) with the α-glucosidase active site, containing one polar interaction and three greasy interactions (**Table 4**). Peptide 8 and acarbose have the same interaction at the amino acid residue Trp425. Three-dimensional visualization of α-glucosidase and peptide 8 is presented in **Figure 4**.

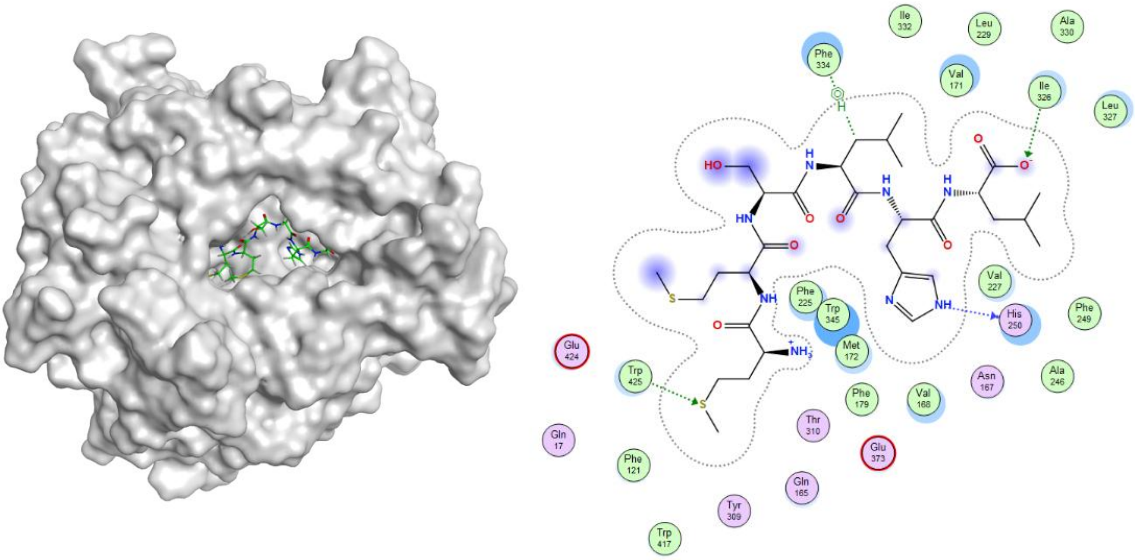

Figure 4. Two- and three-dimensional visualizations of α-glucosidase complex with peptide 8.

Table 4. Type Interactions between ligands and target proteins from molecular docking results

Complex	Hydrophilic interactions			
	Acidic	Basic	Polar	Greasy
Peptide 9-EGFR	Asp800, **Asp855**	**Lys745**, Arg841	Cys797, Gly796	**Leu718**
ATP-EGFR	**Asp855**	**Lys745**	Thr854	**Leu718**, Val726
Peptide 1-AKT1	**Glu228**, Glu234	Lys179, Lys276	-	Val164, Met281
ATP-AKT1	**Glu228**, Glu234, Asp292	-	-	Met227
Peptide 3-VEGFR2	**Asp814**, Asp1046	-	**His1026**	Ala881, Ile1025
ATP-VEGFR2	**Asp814**	-	**His1026**	-
Peptide 8-α-Glucosidase	Glu373, Glu424	Arg312	Gln165, Asn164, His120, Gln17	**Trp425**
ATP-α-Glucosidase	-	-	His250	Ile326, Phe334, **Trp425**
Peptide 1-Glucokinase	**Asp205, Glu290**	-	Thr228, Thr332, Ser411	-
ATP-Glucokinase	**Asp205,** Glu256, **Glu290**	Lys169	Thr168, Asn204, Asn231	-

(-): no interaction
Residues with similar interaction poses with control are bolded.

Molecular docking protein target of glucokinase

Based on molecular docking results, peptides 1, 2, 3, 4, 5, 6, and 10 had RMSD values lower than 2 Å and stronger binding affinity than the Glucokinase inhibitor (-10.37 kcal/mol). The allergenicity and toxicity test showed that peptides 1, 3, and 5 are non-allergenic and non-toxic, with peptide 1 having the lowest binding affinity. Thus, peptide 1 (-9.80 kcal/mol) is the best candidate among all the ten peptides. The 2D interaction analysis showed that peptide 1 forms five interactions (Glu 290, Asp 205, Ser 411, Thr 332, Thr 228), consisting of two acidic and three polar hydrophilic interactions. On the other hand, the glucokinase inhibitor forms seven interactions (Glu 256, Asn 231, Asn 204, Asp 205, Lys 169, Thr 168, Glu 290), consisting of three acidic, one basic, and three polar hydrophilic interactions. Peptide 1 and glucokinase inhibitors formed the same interactions at the amino acid residues Glu 290 and Asp 205 (**Table 4**). The formed glucokinase complex with peptide 1 is visualized and presented in **Figure 5**.

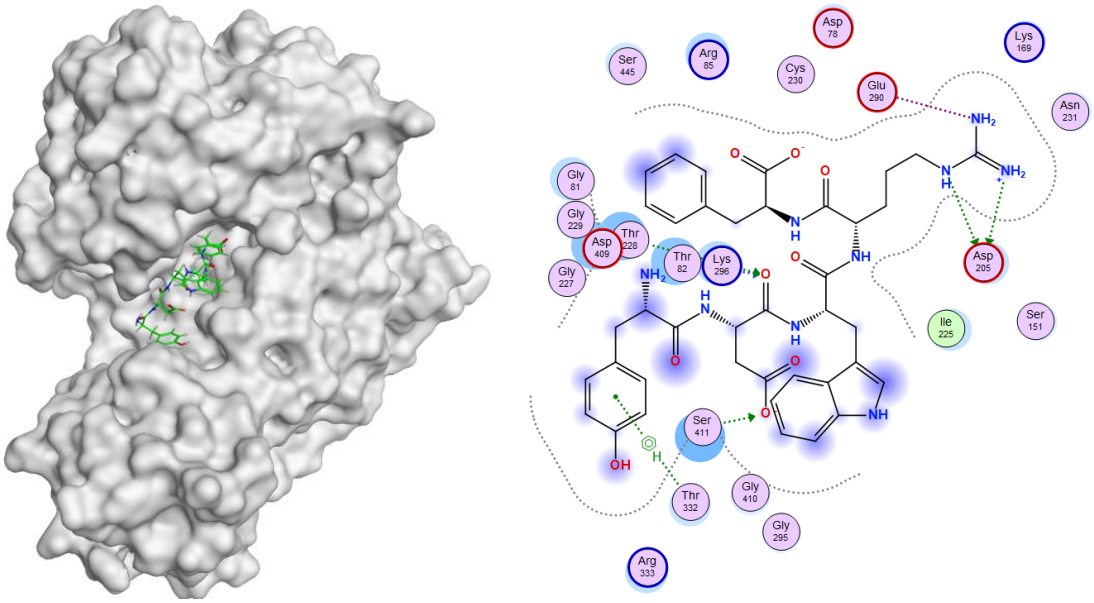

Figure 5. Two- and three-dimensional visualizations of glucokinase complex with peptide 1.

Molecular dynamic simulations

The RMSD values of the simulated complex are presented in **Figure 6**. The RMSD plot shows that all five protein-peptide complexes maintain stability throughout the 50 ns molecular

dynamics simulation, with RMSD values consistently below 3 Å. RMSD values under 3 Å indicate that the molecular complexes are structurally stable over time. Notably, the AKT1-Peptide 1 complex displays the highest stability, with RMSD values consistently below 1.5 Å.

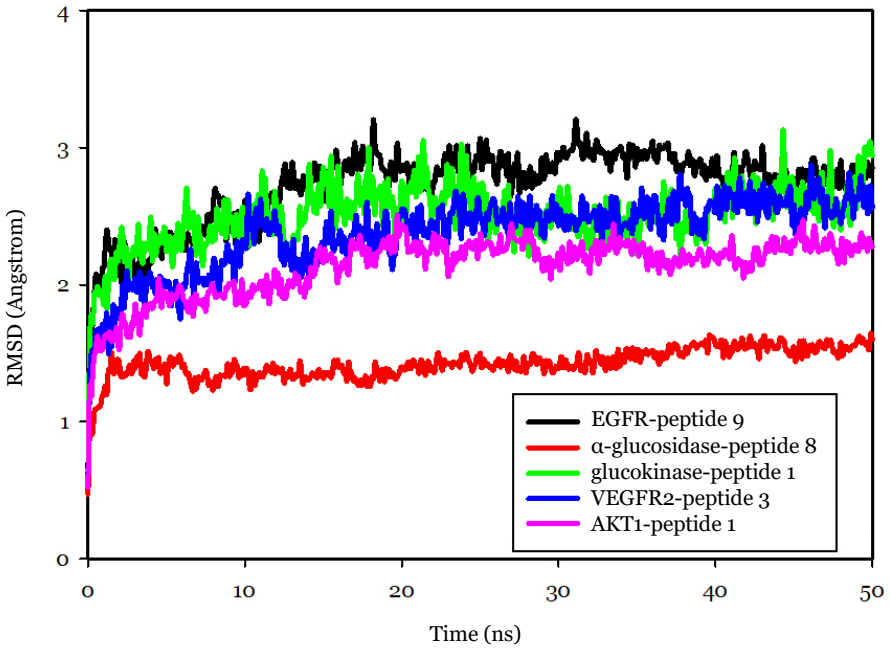

Figure 6. The molecular dynamics simulation of the EGFR-peptide 9, AKT1-peptide 1, VEGFR2-peptide 3, α-glucosidase-peptide 8, and glucokinase-peptide 1. RMSD: root-mean-square deviation.

Discussion

EGFRs are single-chain transmembrane glycoproteins that belong to the Receptor Tyrosine Kinase (RTK) subclass [11]. CTGF/CCN2 (Connective Tissue Growth Factor) is one of the EGFR ligands that is particularly important in kidney disease. This ligand controls renal inflammation, cell proliferation, and fibrosis by binding to EGFR and activating downstream signaling pathways that lead to diabetic nephropathy signs, such as scarring and glomerulus hypertrophy. Transactivation (indirect activation) of EGFR could be provoked by extracellular stimuli, such as TGFB (Transforming Growth Factor Beta), angiotensin II, and other cytokines, demonstrating another significant process in renal pathology [12]. In this in-silico study, peptide 9 of *C. frondosa* celomic fluid was shown to act as a specific ATP competitive inhibitor of EGFR. ATP inhibition can prevent the phosphorylation of EGFR so that the protein is inactive. The reason for choosing the ATP competitive inhibitor strategy is that the ATP binding site is a conserved region that is not easily mutated. Targeting EGFR in nephropathy diabetics has shown promising results in in vivo experiments. Using an EGFR inhibitor combined with erlotinib, the mice showed reduced body weight, fasting blood glucose, islet macrophage infiltration, glomerulosclerosis, and preserved pancreatic insulin levels [13].

The PI3K/Akt signaling pathway is one of the important signaling pathways in regulating DKD development [14]. Activation of this pathway leads to the phosphorylation of Akt, which regulates various cellular processes, including cell survival, proliferation, and metabolism. A previous study showed that inhibiting this pathway prevents high glucose-induced HDAC5 upregulation and TGF-β1 overexpression, indicating its role in the epithelial-mesenchymal transition (EMT) of renal tubular cells in diabetic kidney disease [15]. One strategy is to develop an AKT protein inhibitor [15]. This study showed that peptide 1 specifically inhibits AKT1 activity by blocking ATP binding to the protein. This strategy is expected to prevent the progression of diabetic nephropathy/DKD.

Diabetic nephropathy begins with hyperglycemia that induces angiotensin receptor expression and angiotensin II synthesis by mesangial cells. Angiotensin II stimulates the expression of various protein factors, including VEGF-A, by podocyte cells. An increase in VEGF-

A can lead to increased TGF-beta, endothelial proliferation, and protein accumulation in the glomerular extracellular matrix, leading to kidney damage due to diabetes mellitus or diabetic nephropathy. However, increased VEGF-A can only be affected if it binds to VEGFR-2, the receptor found in podocyte cells [16]. It can be proposed that VEGFR-2 inhibition can suppress the activity of VEGF-A to prevent or reduce the incidence of diabetic nephropathy. A study by Lavoz *et al*., which identified the effect of administering a VEGFR2 inhibitor on the outcome of a mouse model with diabetic nephropathy, showed positive results [17]. Based on this study, it is known that the administration of VEGFR2 inhibitors can improve kidney function, characterized by a decrease in activated VEGFR2 levels, improve albuminuria conditions characterized by a decrease in the albumin/creatinine ratio, and improve microscopic kidney structure characterized by an increase in mesangial matrix and reduce of damaged podocytes [17]. Therefore, the development of VEGFR2 inhibitors as therapy for diabetic nephropathy is highly recommended. Based on this in-silico study, peptide 3 has the potential to act as a specific ATP competitive inhibitor by forming a stronger interaction and similar interactions to ATP in the VEGFR2 ATP binding pocket.

The mechanisms of α-glucosidase, as a carbohydrate hydrolase enzyme, are crucial in regulating blood glucose levels, such as hydrolyzing 1,4-α-glucopyranoside of oligosaccharides and disaccharides to produce monosaccharides [18]. After the digestion process, hydrolyzed glucose is transported through the distribution process by glucose transporter (GLUT)-2 and sodium/glucose cotransporter-1 (SGLT1) from intestinal mucosa into the blood circulation, causing postprandial hyperglycemia (PPHG). Considering α-glucosidase role in elevating glucose absorption in the small intestine during the final stage of glycan catabolism, inhibition of this breakdown process to reduce postprandial hyperglycemia in diabetic patients would be a promising strategy to prevent various diabetic complications [19]. Previous studies have reported that inhibition of α-glucosidase decreases carbohydrate hydrolysis and glucose absorption, stabilizing blood glucose levels and preventing hyperglycemia [20]. In the following study, inhibition of α-glucosidase may slow the hydrolysis rate of maltose and sucrose into glucose, resulting in delayed absorption of intestinal glucose and effectively controlling the acute postprandial blood glucose rapid changes in the in vivo study [21]. Furthermore, α-glucosidase inhibition by LWDH extracts can also reduce fasting blood glucose, TC, TG, LDL-c, cAMP, and HbA1 in diabetic mice [22]. Based on this study, peptide 8 from *C. frondosa* coelomic fluid can inhibit α-glucosidase by binding to its active site. Therefore, *C. frondosa* coelomic fluid peptides not only act as DKD/diabetic nephropathy therapy but also help stabilize blood glucose levels and prevent hyperglycemia.

Glucokinase is an enzyme that plays an essential role in glucose metabolism. The critical role of glucokinase in β-cell glucose metabolism and insulin secretion is highlighted by the fact that inactivating and activating mutations cause diabetes and hyperinsulinism, respectively [23]. Glucokinase activation has been attempted, unsuccessfully, as a therapeutic approach to maintain insulin secretion in diabetes. Paradoxically, glucokinase inhibition, by reducing glycolytic flux, has been shown to preserve β-cell mass, reduce endoplasmic reticulum stress markers, conserve mitochondrial morphology and function, and maintain mature β-cell identity in preclinical mouse models of monogenic diabetes and T2DM [24]. Therefore, inhibiting glucose metabolism using glucokinase inhibitors could be a viable treatment strategy in the early stages of hyperinsulinemia to prevent or delay the eventual β-cell failure that results from the progression of diabetes. Based on the results of this study, peptide 1 from *C. frondosa* coelomic fluid is predicted to act as a specific glucokinase inhibitor. By inhibiting glucokinase activity, the use of *C. frondosa* coelomic fluid peptide is expected to help DKD patients to normalize blood glucose levels and maintain functional β-cell mass.

Conclusion

Based on molecular docking results, *C. frondosa* coelomic fluid peptides have the potential to act as DKD multitarget therapy by inhibiting the phosphorylation of AKT1, EGFR, and VEGFR2. They are also predicted to help DKD patients normalize blood glucose levels, prevent hyperglycemia, and maintain functional β-cell mass by interacting with the active sites of α-

glucosidase and glucokinase. Despite our promising in-silico results, further research (in vitro and in vivo) is necessary to validate therapeutic side effects and efficacy.

Ethics approval

Not applicable.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Not applicable.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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