ORIGINAL ARTICLE

In Silico Analysis Effect of Potential Antidiabetic from Dandang Gendis Extract on Aldose Reductase, Glucokinase, and GSK3β for Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Diabetes mellitus is a metabolic disease characterized by hyperglycemia. Various epidemiological studies have shown a trend of increasing incidence and prevalence of diabetes mellitus in various parts of the world. Therefore, diabetes mellitus is currently a global health threat. Dandang gendis (Clinacanthus nutans) is a widely used plant as a traditional herbal treatment in Indonesia, and it has been proven that the ethanol extract of dandang gendis leaves shows an antidiabetic effect. This research aims to determine the compatibility among the flavonoid compounds in C. nutans with Aldose reductase, glucokinase, and GSK3β target drugs for type 2 diabetes mellitus using in silico method.
Methods: 45 compounds were obtained from multiple sources. The screening method used Lipinski's rule of five and Pyrx until 8 compounds were selected. Avogadro, AutoDock 4.2, and Biovia Discovery Studio 2016 were used for molecular docking and visualization analysis.
Results: Molecular docking results demonstrate that the ligand-protein interaction's binding energy was -7.31 to 35.25 kcal/mol for 1AH3, -7.55 to 0.15 kcal/mol for 1V4S, and -7.99 to -2.85 kcal/mol for 3D0E.
Conclusion: We can conclude that flavonoid compounds Apigenin, Vitexin, 3,3-di-O-Methylellagic Acid, and Clinacoside C show a high binding affinity with Aldose Reductase, Glucokinase, and GSK3β proteins and have the potential to be oral antidiabetic drug compounds for Diabetes Mellitus. However, its binding affinity has not been able to exceed that of the native ligand of the protein. Further research is needed to determine the significant efficacy and potential as an antidiabetic.

Keywords: Clinacanthus nutans, Aldose reductase, Glucokinase, GSK3β, Type 2 diabetes mellitus, In silico, Molecular docking

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Article history: •Received 13 July 2022 •Revised 18 Desember 2022 •Accepted 29 December 2022 •Published 31 January 2023

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia because the body cannot produce enough insulin or use the insulin it does produce effectively (International Diabetes Federation, 2017). According to the International Diabetes Federation (IDF), the number of diabetics in Indonesia will rise from 9.1 million in 2014 to 14.1 million by 2035 (International Diabetes Federation, 2017). WHO also predicts an increase in the number of type 2 DM patients in Indonesia from 8.4 million in 2000 to around 21.3 million in 2030 (The Indonesian Society of Endocrinology, 2021). Numerous studies indicate a global trend toward an increase in the incidence and prevalence of type 2 diabetes (Josten et al., 2006). Diabetes mellitus is classified into type 1 and type 2, with 90% of diabetes cases being type 2 diabetes, which occurs when the body can no longer produce enough insulin to compensate for the increase in insulin resistance (Decroli, 2019). The pathogenesis of type 2 diabetes is based on impaired insulin secretion by pancreatic beta cells and impaired insulin action due to the insensitivity of target tissues to insulin in the muscles and liver (Josten et al., 2006). Persistent hyperglycemia can increase the production of reactive oxygen species (ROS) in the cytosol and mitochondria, thereby supporting the deregulation of antioxidant defenses that can activate various metabolic pathways that will trigger nitro-oxidative stress (NOS) and endoplasmic reticulum stress (Sifuentes-Franco et al., 2017). Protein aldose reductase is a crucial enzyme in the polyol pathway that can lead to the excessive accumulation of ROS (Tang et al., 2012). Glucokinase has a high impact on glucose homeostasis due to its role as a glucose sensor

Available at https://e-journal.unair.ac.id/CIMRJ ; DOI: 10.20473/cimrj.v4i1.42288

in pancreatic cells and as an enzyme-regulating pathway for hepatic glucose clearance and glycogen synthesis (Matschinsky et al., 2011). GSK3Beta is a glycogen synthesis enzyme that is critical in the development of insulin deficiency and resistance (Zhang et al., 2018).

Currently, many recommendations for the treatment of DM involve the use of herbal plants because they have an antihyperglycemic effect by increasing pancreatic performance by increasing insulin secretion or reducing glucose absorption in the intestine (Kooti et al., 2015). Indonesians frequently utilize the plant dandang gendis (Clinacanthus nutans) as a traditional herbal remedy for a variety of ailments, including skin rashes, bug and scorpion bites, diabetes mellitus, fever, and diuretics (Alam et al., 2016). Abdullah and Kasim's research (2017) has proven that the ethanol extract of dandang gendis leaves shows an antidiabetic effect because it contains antioxidants such as flavonoids, saponins, and tannins. Antioxidants are compounds that can reduce the oxidation of molecules by inhibiting the spread of the chain of oxidation reactions (Sifuentes-Franco et al., 2017). Flavonoids are thought to be anti-diabetic due to their modulatory effects on the blood sugar transporter, which include increasing insulin secretion, decreasing apoptosis, and promoting pancreatic -cell proliferation, as well as lowering insulin resistance, inflammation, and oxidative stress in muscle (Vinayagam and Xu, 2015). The use of traditional medicines has become part of Indonesian culture. It has been shown to affect the treatment of diabetes, although more research is needed to ensure the effectiveness and safety of traditional medicines (Dewinta et al., 2020).

Our in silico research would demonstrate the binding site of C. nutans' flavonoid compound to aldose reductase, glucose kinase, and GSK3β proteins. Therefore, this research aims to determine the interaction among the flavonoid compounds in C. nutans with target proteins that are involved in type 2 diabetes mellitus, which are aldose reductase, glucokinase, and GSK3β. The result could be helpful for further research. It is hoped that this research can initiate in vitro and in vivo studies to examine the effect of C. nutans extract in type 2 diabetes mellitus patients.

METHODS

System Configuration

This research was conducted on 2 Windows 10 OS laptops with an Intel® Core i7 processor and Ryzen 7 software with 16 GB of RAM. The applications used in this in silico research are OpenBabel, Pyrx, Avogadro, Autodock 4.2, and Biovia Discovery 2016.

Protein Selection and Preparation

Protein Aldose Reductase (PDB ID: 1AH3), Glucokinase (PDB ID: 1V4S), and GSK3β (PDB ID: 3D0E) are protein targets that will be inhibited for the pathogenesis of type 2 DM. PDB (https://www.rcsb.org/) is a repository for the processing and distributing 3D-structure data of large molecules of proteins and nucleic acids. Most were determined by X-ray crystallography and some by NMR (Samy and Xavier, 2015). 1AH3 and 1V4S proteins only have one protein chain, while 3D0E proteins have two chains, A and B, that form a homodimer. The chain used for the preparation of macromolecules is the A chain. Selected proteins were visualized in Biovia Discovery Studio 2016 to investigate the native ligand and active site for analyzing the grid box. Biovia Discovery Studio 2016 is a single, unified, easy-to-use, graphical interface for robust drug design and protein modeling research (Hariftyani et al., 2021).

Ligand Selection and Preparation

The Pyrx application was used to determine the energy bond between Ligand compounds and the target protein that were selected from ADME (Absorption, Distribution, Metabolism, and Excretion) analysis using Lipinski's rule of five. The ligands' 3D structure was taken from PubChem in the form.sdf. The ligands used for testing their interaction against the above-mentioned proteins were 3,3-di-O-methylellagic Acid, 5-Oxoprolinate, Apigenin, Betulin, Beta Sitosterol, Clinacoside A, Clinacoside B, Clinacoside C, Isovitexin, Lupeol, Stigmasterol, and Vitexin.

Molecular Docking

Ligand optimization was carried out using Avogadro version 1.2 and the Force Field type MMFF94, and the results were saved in.mol2 format. Autodock version 4.2 was used for protein optimization by removing water and other atoms and then adding a polar hydrogen group. Autodock 4.2 was supported by Autodock tools, MGL tools, and Rasmol. The auto grid then determined the native ligand position on the binding site by arranging the grid coordinates (X, Y, and Z). Ligand tethering of the protein was performed by regulating the genetic algorithm (GA) parameters, using 10 runs of the GA criteria (Khaerunnisa et al., 2020). The docking analyses were performed by both Autodock 4.2 and Biovia Discovery Studio 2016. Protein preparation was carried out in the autodock preparation process with each compound. Protein preparation was carried out to separate the native ligand from the target protein and remove H2O. Separation analyses the compound bind to the native ligand site. Previously, the compound or ligand was repaired using the Avogadro application for configuration stabilization.

Before docking the compound with the target protein, the method must be validated to determine the appropriate grid box coordinates. Validation is done by docking between the native ligand and the target protein separated from the native ligand. Proteins must be added with polar-only hydrogens and Kollman charges, while native ligands must be added with polar-only hydrogens, merge nonpolar, and computing gasteiger. The grid box coordinates obtained for protein 1V4S are X.30 Y.34 Z.26 and grid center 40.144 14,796 62,039, protein 3D0E, X.24 Y.36 Z.36 and grid center 20.022 -19.108 7.036, and protein 1AH3, is X. .44 Y.30 Z.16 and grid center 66,868 39,811 88,917. Followed by docking between compounds with other target proteins in the same way, visualization of protein complexes and ligands with 3D and 2D images using the BIOVIA Discovery Studio 2016 application was carried out.

RESULTS

Table 1. Structure of Target Proteins and Active Site Amino Acids (Discovery Studio 2016 and PDB) and Nativel Ligand Structure

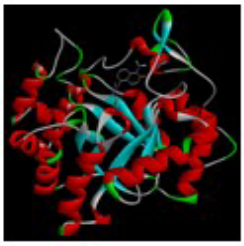
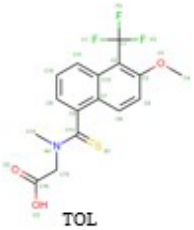
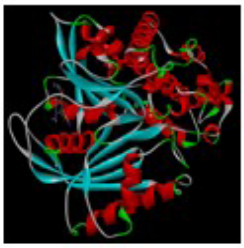
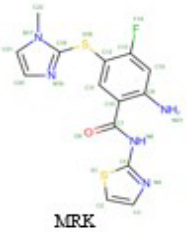
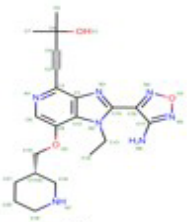
Protein Target	PDB ID	Native Ligand	Active Site
	1AH3	 TOL	CYS303, VAL130, PHE115, LEU300, PHE122, TRP79, TRP111, TYR48
	1V4S	 MRK	ALA456, VAL455, VAL62, TYR61, PRO66, ARG63, ILE159, VAL452, ILE211, TYR214, THR65, SER64, MET235, TYR215
	3D0E	 G93	GLU236, LEU158, VAL166, PHE439, ALA232, ALA179, MET282, GLU230, THR213, MET229, THR292, GLU200, LYS181, PHE163

Table 2. C. nutans Ligand Compounds and ADME Analysis Results

Ligand	CID	Molecular Formula	Lipinski's Rule of 5					TPSA (Å²)	GI Absorption
			Molecule Weight (≤500 g/mol)	H-bond acceptors (≤ 10)	H-bond donors (≤ 5)	LogP ≤ 5	Violation		
3,3-di-O-Methylellagic Acid	5488919	C16H10O8	330.25	8	2	1.90	0	119.34	High
5-Oxoprolinate	21226158	C5H6NO3	128.11	3	1	0.51	0	69.23	Low
Apigenin	5280443	C15H10O4	254.24	4	2	2.27	0	70.67	High
Betulin	72326	C30H50O2	442.72	2	2	4.31	1	40.46	Low
Beta Sitosterol	222284	C29H50O	414.71	1	1	4.79	1	20.23	Low
Clinacoside A	10542082	C10H18O8S	298.31	8	4	0.28	0	141.90	Low
Clinacoside B	10564824	C10H18O7S	282.31	7	4	1.27	0	135.66	Low
Clinacoside C	10830923	C12H21NO8S	339.36	8	5	1.13	0	164.76	Low
Isovitexin	162350	C21H20O10	432.38	10	7	1.94	1	181.05	Low
Lupeol	259846	C30H50O	426.72	1	1	4.68	1	20.23	Low
Stigmasterol	5280794	C29H48O	412.69	1	1	5.01	1	20.23	Low
Vitexin	5280441	C21H20O10	432.38	10	7	1.38	1	181.05	Low

Ligands data (Figure 2) were obtained from and underwent screening through Lipinksi's rule of five and Pyrx application to obtain eight compounds that can be used for docking.

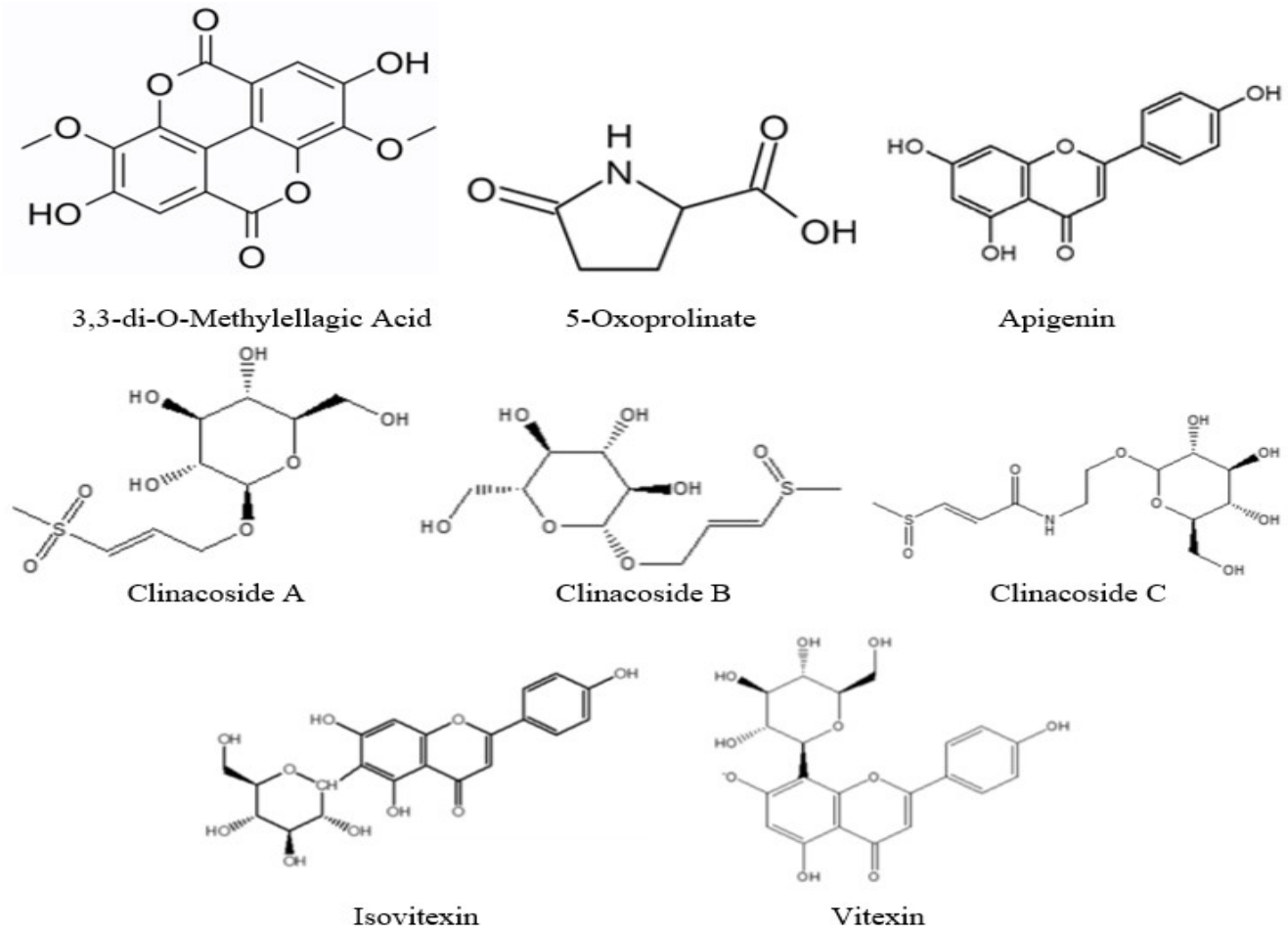
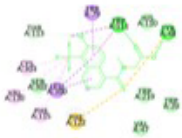
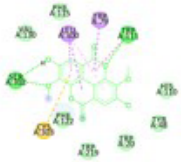
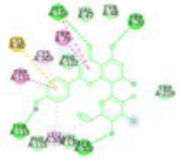
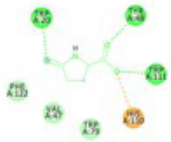
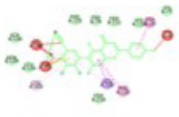
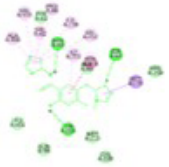
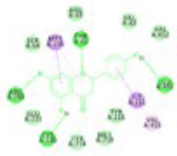
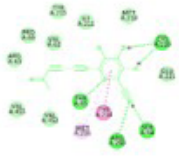
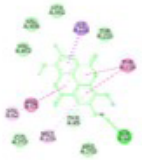
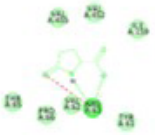
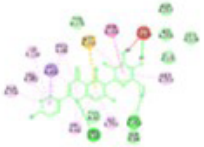


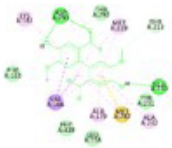
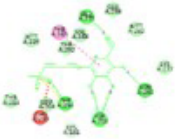
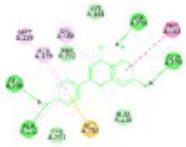
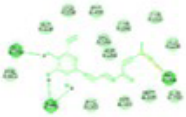
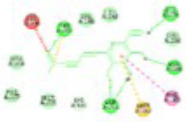
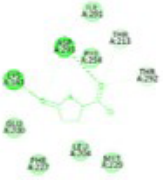
Figure 1. Structures of C. nutans Ligand Compounds

The results of docking using AutoDock are listed in table 3 below. The control used in the docking of three protein analysis is the native ligand-protein itself. From the results of this study, no ligand compound had a lower binding energy than the original protein ligand. Except for isovitexin (35.25 kcal/mol), which tested to have negative binding energy on the target protein, all ligands for protein 1AH3 were discovered. Therefore, all ligands can be considered important as potential drugs (Umadevi). The compound with the lowest binding energy was Apigenin (-7.31 kcal/mol), followed by 3,3-di-O-Methylellagic Acid, Vitexin, Clinacoside A, Clinacoside B, 5-Oxoprolinate, Clinacoside C, and Isovitexin (-7.17 to 35.25 kcal/mol). In 1V4S proteins, all ligand compounds except Vitexin (0.15 kcal/mol) have negative binding energies. Apigenin compound also had the lowest binding energy (-7.55 kcal/mol), followed by Clinacoside C, Clinacoside A, Clinacoside B, 3,3-di-O-Methylellagic Acid, 5-Oxoprolinate, Isovitexin, and Vitexin (-7.53 to 0.15 kcal/mol). 3D0E protein obtained negative binding energy results for all ligand compounds, with Vitexin having the lowest binding energy (-7.99 kcal/mol), followed by 3,3-di-O-Methylellagic Acid, Clinacoside A, Apigenin, Clinacoside C, Clinacoside B, 5-Oxoprolinate, and Isovitexin (-7.02 to -2.85 kcal/mol).

Table 3. Molecular Docking Results and Ligand Visualization of C. nutans with Protein

Protein	Ligand	
1AH3	Native ligand	
		Binding Energy (ΔG) = -9.01
		Ligand Effieciency = -0.38
		Inhibition Constant = 246.71 uM
		Intermolecular Energy = -10.51
		VDW-H Bond Desolvation Energy = -10.36
	Apigenin	
		Binding Energy (ΔG) = -7.31
		Ligand Effieciency = -0.37
		Inhibition Constant = 4.41 uM
		Intermolecular Energy = -8.5
		VDW-H Bond Desolvation Energy = -8.31
	3.3-di-O-Methylellagic Acid	
		Binding Energy (ΔG) = -7.17
		Ligand Effieciency = -0.3
		Inhibition Constant = 5.51 uM
		Intermolecular Energy = -8.37
		VDW-H Bond Desolvation Energy = -8.25
	Vitexin	
		Binding Energy (ΔG) = -5.89
		Ligand Effieciency = -0.19
		Inhibition Constant = 48.31 uM
		Intermolecular Energy = -8.87
		VDW-H Bond Desolvation Energy = -8.72
	Clinacoside A	
		Binding Energy (ΔG) = -5.71
		Ligand Effieciency = -0.3
		Inhibition Constant = 65.27 uM
		Intermolecular Energy = -8.39
		VDW-H Bond Desolvation Energy = -8.31
	Clinacoside B	
		Binding Energy (ΔG) = -5.51
		Ligand Effieciency = -0.31
		Inhibition Constant = 92.13 uM
		Intermolecular Energy = -8.19
		VDW-H Bond Desolvation Energy = -8.11
	5-Oxoprolinate	
		Binding Energy (ΔG) = -4.7
		Ligand Effieciency = -0.52
		Inhibition Constant = 356.38 uM
		Intermolecular Energy = -5.0
		VDW-H Bond Desolvation Energy = -5.05
	Clinacoside C	
		Binding Energy (ΔG) = -3.08
		Ligand Effieciency = -0.14
		Inhibition Constant = 5.54 uM
		Intermolecular Energy = -6.06
		VDW-H Bond Desolvation Energy = -5.91
	Isovitexin	
		Binding Energy (ΔG) = 35.25
		Ligand Effieciency = 1.14
		Inhibition Constant = 32.27 uM
		Intermolecular Energy = 32.27
		VDW-H Bond Desolvation Energy = 32.2

Protein	Ligand	
1V4S	Native ligand	
		Binding Energy (ΔG) = -9.18
		Ligand Effieciency = -0.4
		Inhibition Constant = 187.76 nM
		Intermolecular Energy = -10.37
		VDW-H Bond Desolvation Energy = -10.24
	Apigenin	
		Binding Energy (ΔG) = -7.55
		Ligand Effieciency = -0.38
		Inhibition Constant = 2.9 uM
		Intermolecular Energy = -8.75
		VDW-H Bond Desolvation Energy = -8.49
	Clinacoside C	
		Binding Energy (ΔG) = -7.53
		Ligand Effieciency = -0.34
		Inhibition Constant = 3.01 uM
		Intermolecular Energy = -10.81
		VDW-H Bond Desolvation Energy = -10.58
	Clinacoside A	
		Binding Energy (ΔG) = -6.5
		Ligand Effieciency = -0.34
		Inhibition Constant = 17.24 uM
		Intermolecular Energy = -9.18
		VDW-H Bond Desolvation Energy = -8.97
	Clinacoside B	
		Binding Energy (ΔG) = -6.23
		Ligand Effieciency = -0.35
		Inhibition Constant = 26.96 uM
		Intermolecular Energy = -8.92
		VDW-H Bond Desolvation Energy = -8.69
	3.3-di-O-Methylellagic Acid	
		Binding Energy (ΔG) = -4.52
		Ligand Effieciency = -0.19
		Inhibition Constant = 487.07 uM
		Intermolecular Energy = -5.71
		VDW-H Bond Desolvation Energy = -5.79
	5-Oxoprolinate	
		Binding Energy (ΔG) = -4.02
		Ligand Effieciency = -0.45
		Inhibition Constant = 1.13 mM
		Intermolecular Energy = -4.32
		VDW-H Bond Desolvation Energy = -4.66
	Isovitexin	
		Binding Energy (ΔG) = -0.34
		Ligand Effieciency = -0.01
		Inhibition Constant = 561.73 mM
		Intermolecular Energy = -3.32
		VDW-H Bond Desolvation Energy = -3.22
	Vitexin	
		Binding Energy (ΔG) = 0.15
		Ligand Effieciency = 0.0
		Inhibition Constant = -
		Intermolecular Energy = -2.38
		VDW-H Bond Desolvation Energy = -2.54

Protein	Ligand	
3D0E	Native ligand	
		Binding Energy (ΔG) = -11.09
		Ligand Effieciency = -0.36
		Inhibition Constant = 7.44 nM
		Intermolecular Energy = -13.77
		VDW-H Bond Desolvation Energy = -13.36
	Vitexin	
		Binding Energy (ΔG) = -7.99
		Ligand Effieciency = -0.26
		Inhibition Constant = 7.44 nM
		Intermolecular Energy = -13.77
		VDW-H Bond Desolvation Energy = -10.06
	3.3-di-O-Methylellagic Acid	
		Binding Energy (ΔG) = -7.02
		Ligand Effieciency = -0.29
		Inhibition Constant = 7.11 uM
		Intermolecular Energy = -8.22
		VDW-H Bond Desolvation Energy = -7.73
	Clinacoside A	
		Binding Energy (ΔG) = -6.65
		Ligand Effieciency = -0.35
		Inhibition Constant = 13.43 uM
		Intermolecular Energy = -9.33
		VDW-H Bond Desolvation Energy = -8.97
	Apigenin	
		Binding Energy (ΔG) = -6.39
		Ligand Effieciency = -0.32
		Inhibition Constant = 20.74 uM
		Intermolecular Energy = -7.58
		VDW-H Bond Desolvation Energy = -6.91
	Clinacoside C	
		Binding Energy (ΔG) = -6.09
		Ligand Effieciency = -0.28
		Inhibition Constant = 34.57 uM
		Intermolecular Energy = -9.37
		VDW-H Bond Desolvation Energy = -8.84
	Clinacoside B	
		Binding Energy (ΔG) = -5.95
		Ligand Effieciency = -0.33
		Inhibition Constant = 43.81 uM
		Intermolecular Energy = -8.63
		VDW-H Bond Desolvation Energy = -8.24
	5-Oxoprolinate	
		Binding Energy (ΔG) = -3.97
		Ligand Effieciency = -0.44
		Inhibition Constant = 1.22 mM
		Intermolecular Energy = -4.27
		VDW-H Bond Desolvation Energy = -4.38
	Isovitexin	
		Binding Energy (ΔG) = -2.85
		Ligand Effieciency = -0.09
		Inhibition Constant = 8.1 mM
		Intermolecular Energy = -5.84
		VDW-H Bond Desolvation Energy = -5.57

DISCUSSION

45 ligand compounds from C. nutans were selected from multiple sources. The 15 flavonoid compounds were then chosen for this research. Then the canonical SMILES were taken from PubChem and evaluated with Lipinski's rule of five to predict the solubility and permeability of chemical compounds based on their molecular properties (Bhikshapathi et al., 2010). Drug-like properties using ADME analysis to delineate the relationship between pharmacokinetics and physicochemical parameters (Khaerunnisa et al., 2020). 12 compounds that pass Lipinski's rule of five and are therefore more likely to have the chemical and physical properties to be orally bioavailable, are 3,3-di-O-Methylellagic acid, 5-Oxoprolinate, Apigenin, Betulin, Beta Sitosterol, Clinacoside A, Clinacoside B, Clinacoside C, Isovitexin, Lupeol, Stigmasterol, and Vitexin. 3 compounds did not pass Lipinski's rule of five i.e. Isoorientin, Orientin, and Shaftoside due to having more than two violations so the 3 compounds are non-orally available drugs.

Scientific findings, predominantly experimental studies, have shown that C. nutans extracts possess antihyperglycemic, antioxidant, anti-inflammatory, and antihyperlipidemic effects (Azemi et al., 2020). Flavonoids are the main bioactive compounds in this plant, and different extracts have been found to possess biological activities. Less toxicity of this plant represents its possible uses as a therapeutic remedy for several ailments (Alam et al., 2016). Aldose reductase belongs to the aldoketo reductase superfamily. It is the first rate-limiting enzyme in the polyol pathway and reduces glucose to sorbitol by utilizing NADPH as a cofactor. Sorbitol dehydrogenase is the enzyme responsible for converting sorbitol into fructose (Madeswaran et al., 2012). The glucose-phosphorylating enzyme glucokinase has a crucial role in glucose homeostasis as 'glucose sensor' of the insulin-producing pancreatic β-cells and as a regulatory step in the conversion of glucose to glycogen, as well as in gluconeogenesis in the liver (Matschinsky et al., 2011). GSK-3β plays a crucial role in regulating blood glucose, which is the primary rate-limiting enzyme for inhibition of glycogen synthesis (Zhang et al., 2018).

Molecular docking results establish that all ligands successfully bind to the proteins' active sites. The interactions between ligands and amino acids are visualized in 2D diagrams (Table 3), which describe that all C. nutans ligand compounds that we docked could provide solid and stable complexes to 1AH3, 1V4S, and 3D0E. Native ligand binding sites in 1AH3, 1V4S, and 3D0E proteins were mentioned in Table 1. The binding site of Apigenin in protein 1AH3 was found to be LEU300, TYR48, TRP111, TRP79, TRP20, SER302, while in 1V4S was MET235, THR65, LEU451, ILE211, VAL455, CYS220, and ARG250. This proves that the effective binding sites are present in the selected ligand compared with the native ligand. It demonstrates Apigenin's ability to inhibit the enzymes Aldose reductase and glucokinase. Apigenin compounds can act as antioxidants, anti-cancer, and anti-inflammatory. Apigenin as an anti-inflammatory compound acts as a protective agent in several disorders through inhibiting inflammatory, pathway, and molecular mediators. Apigenin compounds can suppress p65 phosphorylation in monocytes/macrophages and reduce COX-2 and NO expression in mouse macrophages. It can minimize inflammation-derived obesity and relieve muscle and liver steatosis (Ginwala et al., 2019). In another study, Apigenin was assessed to lower glucose in mice by acting as an anti-cancer. Administration of Apigenin can reduce hepatic glucose-6-phosphate levels and serum cholesterol levels which generally increase in diabetes mellitus patients (Panda and Kar, 2007).

3,3-di-O-Methylellagic acid showed that this compound might exert an anti-diabetic effect by suppressing the absorption of carbohydrates from the gut, thereby reducing the postprandial rise in blood glucose (Tabopda et al., 2008). 3,3-di-O-Methylellagic acid also showed an inhibitory effect on glucose transport assays (Bai et al., 2008). The binding site for the compound 3,3-di-O-Methylellagic Acid on protein 1V4S was found at ILE211, TYR214, SER64, MET235, ARG63, VAL62, in protein 1AH3 were found LEU300, TRP79, TRP111, SER302, CYS303 and in protein 3D0E was LYS181, ASP293, MET229, GLU230, ALA232, MET282, ALA179, VAL166. This proves that 3,3-di-O-Methylellagic Acid binds to the active protein sites Glucokinase, Aldose Reductase, and GSK3β. 3,3-di-O-Methylellagic Acid compound obtained the lowest binding energy value in 1V4S protein and the second-lowest after Apigenin in 1AH3 and 3D0E proteins, so it can be concluded that the 3,3-di-O-Methylellagic Acid compound can inhibit all three proteins.

Clinacoside C obtained the second lowest binding energy value in 1V4S proteins, with binding site results being ARG63, ILE211, MET235, TYR214, SER64, GLU221, CYS220, MET210, TYR61. Clinacoside C also has a negative binding energy value for 1V4S and 3D0E proteins but below other compounds. Vitexin increases pancreatic antioxidant enzymes and promotes regeneration (Nurdiana et al., 2017). Vitexin showed significant inhibition of glucose or methylglyoxal-induced AGEs formation with more than 85% efficacy at 100 M (Peng et al., 2008). Vitexin, the main bioactive flavonoid compound in plants, has many pharmacological properties, including antioxidant, anti-inflammatory, and anti-myeloperoxidase. In the study of Wang et al. (2017) (Wang et al., 2017), Vitexin decreases apoptosis and damage caused by lipopolysaccharides in mouse islet tissue and INS 1 cells. Apoptosis is considered one of the mechanisms that contribute to the inflammatory response of cells in DM. In addition, pre-treatment with Vitexin decreased the levels of mouse proinflammatory cytokines tumor necrosis factor, and HMGB1 in LPS-induced mice.

Further studies demonstrated that vitexin pre-treatment suppressed the activation of the P38 mitogen-activated protein kinase signaling pathway in LPS-induced INS-1 cells (Wang et al., 2017). In 3D0E proteins, vitexin had the highest binding energy value and binding site results at MET282, GLU236, GLU279, ASN280, ASP293, LYS181, GLU200, ALA179, MET229, THR292, and VAL166. Vitexin obtained a positive binding energy value on 1V4S protein and a negative binding energy value on 1AH3 protein, but it was still below other compounds. So it can be concluded that Vitexin can inhibit the GSK3β and Aldose Reductase enzymes but does not inhibit the Glucokinase enzyme.

CONCLUSION

Finally, the results of in silico molecular docking show that Apigenin, 3,3-di-O-Methylellagic Acid, Clinacoside C, and Vitexin have high binding affinity for Aldose reductase, Glucokinase, and GSK3 protein. These flavonoids derived from C. nutans have the potential to be oral antidiabetic drug compounds for Diabetes Mellitus due to their drug-likeness based on Lipinski's rule of five. However, the binding affinity of C. nutans' flavonoid has not been able to exceed the native ligand of the protein. Therefore, further research is

required to investigate the significant efficacy and potential of the compounds above as antidiabetic agents, such as in vitro, in vivo, and clinical trials.

ACKNOWLEDGEMENT

The author thanks the parties involved in completing this study.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

ETHICS CONSIDERATION

This research was not applicable for ethically cleared.

FUNDING DISCLOSURE

This research was self funded.

AUTHOR CONTRIBUTION

All author have contributed to all procss in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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