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Potential of cocoa (*Theobroma cacao*) shell for diabetic neuropathy targeting transient receptor potential canonical (TRPC): An *in silico* study

Maryo Adjie Pangestu, Sarmoko*, I Gede Raditya Purwanata, Titah Zusela

Department of Pharmacy, Faculty of Science, Institut Teknologi Sumatera, South Lampung 35365, Indonesia

*Corresponding author: Sarmoko, Jl. Terusan Ryacudu, Way Huwi, Jati Agung, South Lampung 35365, Lampung, Indonesia. Email: sarmoko@fa.itera.ac.id

Abstract: Diabetic neuropathy, a painful complication of diabetes mellitus, may potentially be treated with compounds found in cocoa pods. This study investigates the interactions of various flavonoids (catechin, epicatechin, quercetin, luteolin, apigenin, naringenin, and procyanidin) contained in the cocoa pod to the Canonical Transient Receptor Potential (TRPC6) receptor. Molecular docking, facilitated by Autodock software, was employed to predict the binding affinities of these compounds to TRPC6. This involved preparing the molecular structures of the flavonoids and the TRPC6 protein for simulation. The simulation provided insights into the binding efficiencies and interaction energies between the flavonoids and TRPC6. The findings indicate that procyanidin and quercetin exhibit the highest binding energies, at -7.15 kcal/mol and -6.37 kcal/mol, respectively. Procyanidin interacts with the amino acid residues Ala508, Arg609, Arg758, Asn765, Asp530, Glu512, His446, and Met505, while quercetin binds to Arg758, Asp530, Glu512, and Glu524. These results highlight the potential of quercetin and procyanidin as candidates for the development of TRPC6-targeted treatments for diabetic neuropathy. This study lays the groundwork for the creation of new, effective, and safe diabetic neuropathy medications.

Keywords: diabetic neuropathy, TRPC6, molecular docking, flavonoids

Introduction

Diabetes mellitus (DM) is a chronic condition and one of the top causes of adult mortality, responsible for 4 million deaths worldwide in 2017. According to the International Diabetes Federation (IDF), the number of people with diabetes has increased significantly over the years: 285 million in 2009, 366 million in 2011, 382 million in 2013, 415 million in 2015, 425 million in 2017, and 463 million in 2019 [1]. Diabetic neuropathy is a prevalent complication among individuals with diabetes mellitus. Approximately 371 million people globally are diagnosed with diabetes, with about 60-70% of them experiencing diabetic neuropathy complications [2,3].

Transient receptor potential canonical (TRPC) channels are a group of non-selective cation channels that allow calcium permeability and are regulated by receptors within the TRP superfamily. Mammals have seven TRPC members, classified into four subgroups (TRPC1, TRPC2, TRPC4/5, and TRPC3/6/7) based on their amino acid sequences and functional similarities [4]. These channels are vital for various cellular and physiological functions. It has been found that TRPC6 protein expression influences neuropathic pain in diabetic rats, with increased TRPC6 expression observed in diabetic rats [5,6]. The primary treatments for diabetic neuropathy include pregabalin and gabapentin [7]. Gabapentin and pregabalin can be effective in reducing neuropathic pain, but they do not address oxidative stress. To tackle the underlying oxidative stress in diabetic neuropathy, additional interventions that specifically target oxidative imbalance in the body are needed [8].

Flavonoid compounds are known to help in preventing diabetes and its complications. Flavonoids are known to help reduce oxidative stress and may have the potential to inhibit GLUT2 in the intestinal mucosa, thereby decreasing glucose and fructose absorption. Additionally, flavonoids can increase cAMP in pancreatic beta cells by inhibiting phosphodiesterase, leading to enhanced insulin secretion through the release of protein kinase A (PKA) [9,10]. Cocoa (*Theobroma cacao* L.) contains several flavonoid compounds, such as catechin, epicatechin, quercetin, luteolin, apigenin, naringenin, and procyanidin. Previous research indicates that cocoa flavonoids have a beneficial role in reducing the risk of

Table 1. Macromolecule validation

Macromolecule	Ligand	Grid coordinates	**Binding energy (kcal/mol)**	**RMSD (Å)**
7DXG	HOR	X = 137.723 Y = 110.221 Z = 152.409	-10.96	0.80

Table 2. Ligand selection based on Lipinski's rules

Metabolite	**MW (g/mol)**	**Log P**	**HBA**	**HBD**	**Violations**	**GI absorption**
Apigenin	270	2.4	5	3	Yes; 0 violation	High
Epicatechin	290	1.54	6	5	Yes; 0 violation	High
Catechin	290	1.54	6	5	Yes; 0 violation	High
Luteolin	286	2.12	6	4	Yes; 0 violation	High
Naringenin	272	2.50	5	3	Yes; 0 violation	High
Procyanidin	594	2.73	13	10	Yes; 3 violation	Low
Quercetin	302	2.01	7	5	Yes; 0 violation	High

neurodegenerative diseases, including nephropathy in diabetic patients [11].

However, there is limited research on the potential of cocoa shell for diabetic neuropathy. Therefore, we are interested in exploring the potential of cocoa shell for diabetic neuropathy targeting TRPC through an *in silico* study using molecular docking. This research aims to determine the binding energy of compounds found in cocoa shell (catechin, epicatechin, quercetin, luteolin, apigenin, naringenin, and procyanidin) to the TRPC6 protein through molecular docking.

Methods

This study uses the structural data of flavonoid compounds and the TRPC6 protein (obtained from RCSB PDB, PDB ID: 7DXG). The independent variables include catechin, epicatechin, quercetin, luteolin, apigenin, naringenin, and procyanidin. The dependent variable is the transient receptor potential canonical 6 (TRPC6). The constant variables are BIOVIA Discovery Studio, AutoDock 4.2.6, PubChem, and the Protein Data Bank.

The TRPC6 protein was retrieved from http://www.rcsb.org/. The preparation of the target protein was done using Chimera 1.11.1 by removing the original ligand 4-[[(1R,2R)-2-[(3R)-3-azanylpiperidin-1-yl]-2,3-dihydro-1H-inden-1-yl]oxy]-3-chloranyl-benzenecarbonitrile (HOR).

Docking validation was carried out using AutodockTools (Autodock 4.2 and Autogrid). The grid box was configured with center coordinates (X = 137.723, Y = 110.221, Z = 152.409) and grid size (X = 0.375 Å, Y = 0.375 Å, Z = 0.375 Å). The Root Mean Square Deviation (RMSD) value, a parameter for validating molecular docking methods, is considered valid if it is less than 2.0 [12].

The 3D structures of catechin, epicatechin, quercetin, luteolin, apigenin, naringenin, and procyanidin were downloaded from https://pubchem.ncbi.nlm.nih.gov/ and optimized using HyperChem 8. The optimization was performed with the semi-empirical AM1 (Austin Model 1) method, including single-point calculations and geometric optimization [13].

The optimized compounds of catechin, epicatechin, quercetin, luteolin, apigenin, naringenin, and procyanidin were docked into the TRPC6 protein using AutoDock 4.2.6. The docking was conducted with the same grid box dimensions used for validation. The results will display the conformations with the lowest binding energy for interaction with the target protein. To illustrate the ligand-receptor interactions, BIOVIA Discovery Studio software was employed [12].

The results are analyzed based on the binding free energy (ΔG), which reflects the strength of the ligand-receptor interaction. A lower or more negative ΔG indicates a stronger and more stable binding between the ligand and the receptor. These findings could offer alternative approaches to treating diabetic neuropathy using natural substances [14].

Table 3. Molecular docking of compounds binding to TRPC6 (PDB ID 7DXG)

Metabolite	Binding energy (kcal/mol)	Number of Hydrogen Bonds	Interactions with Amino Acids of Hydrogen Bonds
Apigenin	-5.18	3	Ile610, Val603, Val604
Epicatechin	-5.80	4	Arg609, Asp530, Glu509, Glu524
Catechin	-5.77	4	Ala508, Asp530, Glu512, Glu524
Luteolin	-6.14	4	Arg758, Asn527, Asp530, Glu524
Naringenin	-5.27	4	Arg609, Arg758, Glu509, Glu512
Procyanidin	-7.15	8	Ala508, Arg609, Arg758, Asn765, Asp530, Glu512, His446, Met505, Tyr612
Quercetin	-6.37	4	Arg758, Asp530, Glu512, Glu524

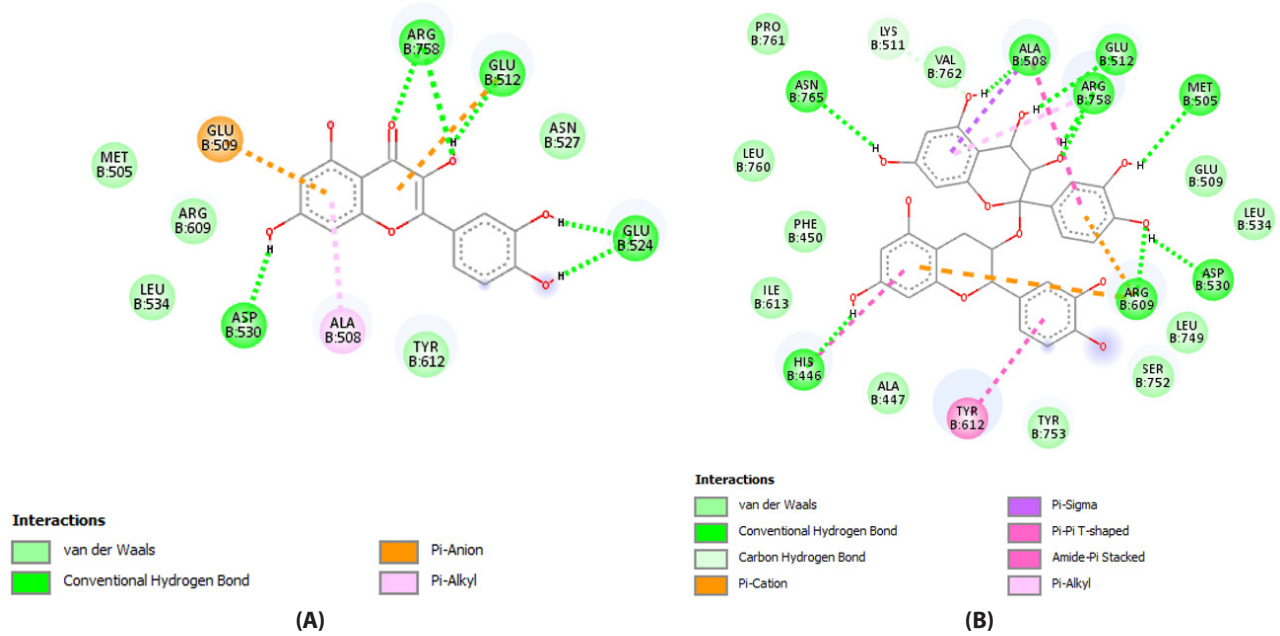

Figure 1. Visualization of the 2D interactions of compounds to TRPC6 (PDB ID 7DXG). (A) Quercetin, (B) Procyanidin

Results

Protein validation was performed through re-docking of the native ligand HOR with the TRPC6 receptor. The results of the validation are shown in Table 1.

The seven compounds to be used were selected based on Lipinski's rules. The selection results are shown in Table 2.

The selected and optimized compounds were docked to the active site of the protein during the validation process. The docking results are shown in Table 3.

Discussion

This research aims to gather information about flavonoid compounds (catechin, epicatechin, quercetin, luteolin, apigenin, naringenin, and procyanidin) found in cacao shell, which could potentially serve as an alternative treatment for diabetic neuropathy targeting TRPC6. The study employs molecular docking techniques to assess the interactions between these flavonoid compounds and the TRPC6 target. The Autodock software is utilized to perform the docking and predict the binding affinity between the compounds and TRPC6.

Protein preparation and the validation of natural protein-ligand interactions are achieved using AutoDock 4.2.6. This process involves removing non-amino acid residues and water molecules. Additionally, macromolecule optimization includes adding hydrogen atoms and Kollman charges [15]. These modifications ensure the macromolecule's compatibility with the

human body, maintaining charge and atomic structure [16]. Protein validation is conducted through re-docking of the native ligand with its protein, HOR, and the TRPC6 receptor. The RMSD value is used as a parameter to verify the accuracy of the re-docking results [12]. According to Table 1, the 7DXG validation achieved an RMSD of 0,80 Å, meeting the criterion of RMSD < 2 Å. Consequently, the 3D coordinates of the protein's active site can be utilized for docking with ligands from bioactive compounds in cacao shell.

A review of the bioactive compounds in cacao was conducted through literature research. Following Lipinski's principles, which assess drug-like properties, attributes such as molecular weight, log partition coefficient, hydrogen bond donors, and acceptors are considered. Lipinski's rule of five suggests that a compound with a molecular weight less than 500 Dalton is preferable, as larger molecules may struggle to cross cell membranes. A higher molecular weight increases hydrophobicity, and the log P coefficient should be <5 to indicate adequate fat/water solubility. Hydrogen bond donors (HBD) should be <5, and hydrogen bond acceptors (HBA) should be <10, as excessive hydrogen bonding can hinder absorption [17,18].

Based on the docking results presented in Table 3, six active compounds—apigenin, epicatechin, catechin, luteolin, naringenin, and quercetin—show strong potential as inhibitors of the TRPC6 molecular target. This suggests that these cacao-derived bioactive compounds could be promising candidates for developing new treatments for diabetic neuropathy.

The compounds exhibiting the most effective activity against the target protein are procyanidin and quercetin. Procyanidin, a flavonoid, shows a binding energy of -7.15 kcal/mol and interacts with residues Ala508, Arg609, Arg758, Asn765, Asp530, Glu512, His446, Tyr612, and Met505. Quercetin, with a binding energy of -6.37 kcal/mol, interacts with residues Arg758, Asp530, Glu512, and Glu524 (Figure 1). Hydrogen bonds, which are prevalent in biological systems like proteins and nucleic acids [19], play a key role in maintaining protein stability.

The presence of hydrogen bonds in molecular simulations indicates that these interactions are stable. Binding energy and hydrogen bonding are critical outcomes of molecular docking, with lower binding energy suggesting a stronger and more stable interaction between the ligand and the protein. This implies that procyanidin and quercetin interact effectively with TRPC6, forming hydrogen bonds with key active site residues such as Arg758, Tyr753, Tyr612, His446, Ser752, and Arg609 [20]. Consequently, the active sites of procyanidin and quercetin closely resemble those of the native ligand, and their affinity for TRPC6 is comparable to that of the native ligand, making them effective inhibitors.

Conclusion

The in silico molecular docking study and ligand-protein interaction analysis indicate that the selected active compounds—apigenin, epicatechin, catechin, luteolin, naringenin, procyanidin, and quercetin—demonstrate strong potential as inhibitors of the TRPC6 target. Among these, procyanidin and quercetin exhibit the highest activity. Procyanidin has a binding energy of -7.15 kcal/mol with residues Ala508, Arg609, Arg758, Asn765, Asp530, Glu512, His446, and Met505, while quercetin has a binding energy of -6.37 kcal/mol with residues Arg758, Asp530, Glu512, and Glu524. It is recommended that both this study and future research include in vitro and in vivo tests of these bioactive compounds to confirm their effectiveness against TRPC6.

The compounds apigenin, epicatechin, catechin, luteolin, naringenin, and quercetin meet Lipinski's rule of five, suggesting that they have favorable drug-like properties and are likely to have good absorption and distribution profiles in the body.

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Declaration of interest

The authors declare no conflict of interest.

Author contributions

MAP conducted the literature review, consulted with the supervisor, and performed data analysis. IGRP carried out the molecular docking studies. TZ was responsible for manuscript writing. SM provided

guidance, designed the study, and coordinated the final manuscript preparation.

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