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Research Article
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Molecular Docking of Pomegranate Peel (*Punica granatum* L.) Active Compounds by Targeting Tumor Necrosis Factor Alpha (TNF-α) in Diabetes Condition

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ABSTRACT: *Diabetes mellitus is a group of metabolic disorders characterized by increased blood glucose concentration, known as hyperglycemia. Several studies report that diabetes mellitus is associated with the presence of tumor necrosis factor-α (TNF-α). One of the medicinal plants that has the potential to be used to treat diabetes mellitus is pomegranate (Punica granatum L.). Therefore, this study aimed to observe the effect of bioactive compounds of pomegranate peel as antidiabetic against TNF-α using a molecular docking approach. The stages of molecular docking include geometry optimization, validation of the docking program, docking ligand test, and visualization of the docking results. The target compounds analyzed from pomegranate peel were ellagitannin and punicalagin. The macromolecular target wasTNF-α. Redocking between the original ligand and the target TNF-α showed an RMSD value of ≤ 2.0 Å with a value of 0.4902 Å. The hydrophobic interaction was formed between ellagitannin and punicalagin on the TNF-α target shows excellent ligand-receptor interactions, this can be seen from the binding free energy value which is smaller when compared to the co-crystal ligand. These results indicate that the complex formed from tethering ellagitannin and punicalagin to the TNF-α target is more stable than the original ligand complex with the TNF-α target. Based on the research results, it can be concluded that pomegranate peel has excellent potential as an antidiabetic. In silico research shows that pomegranate peel content such as ellagitannin can bind stably with TNF-α (ΔG = -132.44kcal/mol), so it is predicted to be able to suppress TNF-α which is responsible for the emergence of diabetes mellitus.*

Keywords: *diabetes; peel; pomegranate; TNF-α.*

1. Introduction

Diabetes mellitus is a group of metabolic disorders characterized by increased blood glucose concentration, known as hyperglycemia. According to the International Diabetes Federation (IDF), in 2017, the estimated number of adults living with diabetes increased to 451 million (age 18-99 years) in the world. New projected data suggest that by 2045 this condition will increase to 693 million people with diabetes mellitus [1,2]. Several studies report that diabetes mellitus is associated with the presence of tumor necrosis factor-α (TNF-α) [3-5].

TNF-α is a cytokine secreted by macrophages and other cells such as adipocytes. This cytokine is responsible for systemic inflammation and can stimulate acute phase reactions. TNF-α can inhibit insulin transduction and affect glucose metabolism, which has implications for metabolic disorders such as the emergence of type 2 diabetes mellitus (T2DM) [6,7]. So far, T2DM has been treated using non-pharmacology and pharmacology therapy. However, long-term use of synthetic drugs will certainly cause side effects. Thus, the use of natural products can be considered for development as drug candidates. One plant that has the potential to be developed as an antidiabetic candidate and is often found in Indonesia is pomegranate.

Pomegranate, scientifically known as *Punica granatum* L., is a great source of bioactive substances, primarily polyphenols. Although this plant originated in central Asia, it is now planted across the Mediterranean basin, Asia, and California in the United States due to its exceptional adaptability to a variety of climatic and soil conditions. Since ancient times, pomegranate fruit has been widely used in traditional medicine across various cultures, including Greek, Ayurvedic, Unani, and Egyptian [8]. Considered as "super fruit," it has an abundance of antioxidants and phytochemicals and is linked to numerous health advantages [9-13].

Peels from pomegranates are frequently regarded as agricultural waste. Recent research, however, indicates that it has the potential to be a rich source of bioactive substances with a variety of pharmacological effects. Pomegranate peel's remarkable bioactivity is attributed to its polyphenol, dietary fiber, and vitamin contents. Due to the presence of phytochemicals including gallic acid, ellagic acid, and punicalagin, research has demonstrated several pharmacological effects from pomegranate peels, including cardiovascular protection, anti-cancer, antimicrobial, wound healing, and anti-inflammatory [14]. Therefore, this study aims to predict the effect of bioactive compounds from pomegranate peel (especially, ellagitannin and punicalagin) in silico targeting TNF-α.

2. Methods

2.1. Instrument

The hardware used for the in silico procedure was an ASUS A456U notebook (Operating System: Microsoft Windows 10 Pro 64-bit; Memory: 4 GB; Processor: Intel Core i5-7200U). Meanwhile, the software used is Avogadro 1.2.0, Chimera 1.17.3, Discovery Studio 2016, iGemdock 2.1, and Orca 5.0.3. [15].

2.2. Materials

The test compounds ellagitannins and punicalagin were obtained by downloading the 3D structure via the Pubchem page (https://pubchem.ncbi.nlm.nih.gov/). In the molecular docking study, the macromolecule used as the target was TNF-α (PDB:2AZ5) which was downloaded from PDB (https://www.rcsb.org) in complex form with its co-crystal ligand (trifluoromethylphenyl indole and dimethyl chromone).

2.3. Geometry optimization

The test compounds ellagitannins and punicalagin were optimized with Avogadro. Next,

geometry optimization was carried out for both structures using the semi-empirical PM3 method using Orca. The geometric optimization results of the two compounds were then converted and saved in pdb file format.

2.4. Validation of the docking program

The docking program was validated using natural ligand redocking on the TNF-α target (PDB:2AZ5) using iGemdock [16]. Next, the root mean square deviation (RMSD) value of the best ligand pose resulting from the redocking was calculated. The expected output was to obtain a method that produces a docking pose with the lowest RMSD value (cut-off value < 2.0 Å) [17].

2.5. Docking ligand

Molecular docking was carried out using the method selected from the redocking validation results. Docking was carried out on the TNF-α target with tannin compounds contained in pomegranate peel (*Punica granatum* L.), namely ellagitannins and punicalagin, as ligands for the compounds tested.

2.6. Analysis and visualization of docking results

Next, the docking results were analyzed by observing the free energy value (ΔG) of the best docking results and then comparing the docking poses between the test ligand complexes of ella–gitannins and punicalagin with natural ligands using Discovery Studio 2016.

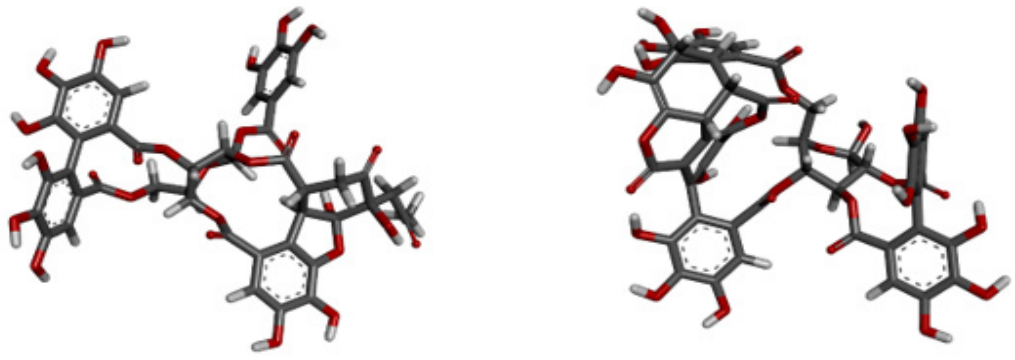
3. Result and discussions

3.1. Geometry optimization

In this study, geometry optimization was performed on the test compounds, specifically ellagitannins and punicalagin, using the semi-empirical PM3 method to obtain their 3D structures. PM3 method, is a semi-empirical quantum mechanical calculation technique and geometry optimization, was performed for all compounds. It is long known for its intermediate accuracy and speed especially when dealing with organic molecule optimizations. This method uses empirically derived parameters to replace complex integrals and thus simplifies the calculations, but also introduces some level of inaccuracy. This not with standing, when optimized on these, it has outperformed calculation protocols for certain types of substances (like organic compounds) [18]. The test compounds are tannin molecules (ellagitannins and punicalagin) classified as organic compounds. Figure 1 are the results of the geometry optimization for the two test molecules to obtain their 3D structures.

3.2. Validation and docking simulation

From the redocking results between the co-crystal ligand and the target TNF-α, the RMSD value ≤ 2.0 Å was 0.4902 Å. The RMSD value from the redocking results shows that the iGemdock software can place the co-crystal ligand position back in its position with a shift from the original



(a) (b)

Figure 1. 3D structures of (a) Ellagitannins and (b) Punicalagin

position between the co-crystal ligand crystallography result and the redocking result ≤ 2 Å, thus meeting the valid criteria. RMSD overlay can be seen in Figure 2.

The hydrophobic interaction formed between ellagitannins and punicalagin on the TNF-α target can stabilize the ligand-receptor interaction which can be seen from the binding free energy value which is smaller when compared to the co-crystal ligand (Table 1). This can be seen from the visualization of the results of tethering the TNF-α target, which has many hydrophobic interaction features with amino acid residues in the enzyme's active site compared to the co-crystal ligand. Apart from that, from the visualization results, ellagitannins and punicalagin also have hydrogen bonds with several amino acid residues found on the active site of the TNF-α target. These interactions are not present in the co-crystal ligand interactions with amino acid residues in the active site of the TNF-α target. The number of hydrogen bonding interaction features possessed by the ellagitannins and punicalagin complex on the TNF-α target also correlates with the binding free energy (ΔG) value, where the binding free energy (ΔG) for ellagitannins and punicalagin is lower when compared to native the ligand. These results indicate that the complex formed from the tethering of ellagitannins and punicalagin to the TNF-α target is more stable than the co-crystal ligand complex with the TNF-α target. We therefore introduced an assessment of the conformation with which both test compounds dock at their binding site. This evaluation was conducted by comparing co-crystal ligand vs derived compound conformation, from their residue interactions similarity. Figure 3 further supports this at the punicalagin ligand (red), due to its orientation that is significantly different from both co-crystal ligand conformation (green) and ellagitannins (blue) compound toward the outside of the active site.

4. Conclusion

The research results show that the complex of ellagitannins and TNF-α demonstrated increased hydrogen bonds and hydrophobic interactions at the active site, conferring lowered binding free energy (ΔG =-132.44kcal/mol) in favor of a more favorable conformation. This demonstrates that ellagitannins have a potential as TNF-α inhibitors.

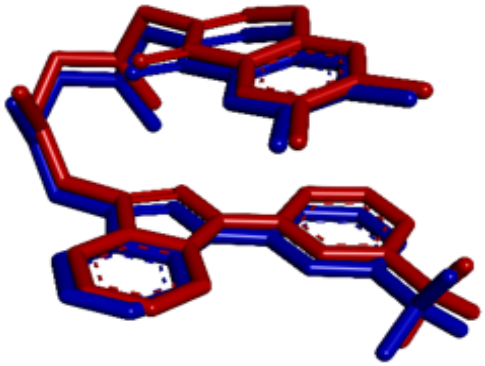


Figure 2. RMSD overlay co-crystal ligand (blue color shows before redocking; red color after redocking)

Table 1. Docking of ellagitannins and punicalagin on TNF-α targets

Compounds	ΔG (kcal/mol)	Hydrophobic interaction	Hydrogen bond
Ellagitannins	−132.44	Tyr119	Tyr119, Tyr151, Gly121
Punicalagin	−125.44	Tyr119, Leu120, Ile155	Ser60, Gln61, Tyr151
Co-crystal ligand	−114.37	Tyr59, Tyr119, Gly121, Tyr151	-

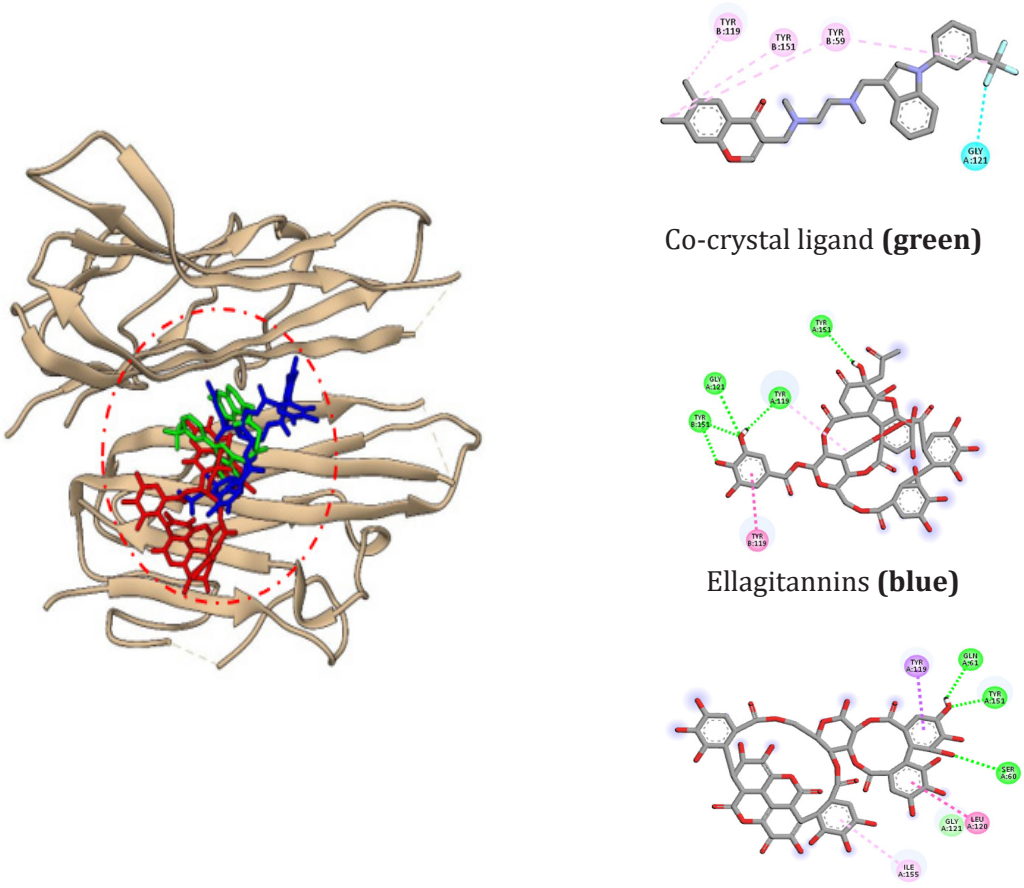


Figure 3. Visualization of ellagitannins and punicalagin at the active sites of TNF-α targets

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References

1. Cho NH, Shaw JE, Karuranga S, et al. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice*. 2018;138:271-281.
2. Al-Lawati JA. Diabetes mellitus: A local and global public health emergency!, *Oman Medical Journal.* 2017;32(3):177-179.
3. Lestari IC, Lindarto D, Ilyas S, et al. Effect of *Phaleria macrocarpa* (Scheff.) Boerl leaf ethanol extract on serum IL-6 and TNF-α levels in diabetic rats. *Med Arch*. 2023;77(4): 254-257.
4. Mardiah, Zakaria FR, Prangdimurti E, Damanik R. Anti-inflammatory of purple roselle extract in diabetic rats induced by streptozotocin. *Procedia Chemistry*. 2015;3:182-189.
5. Syiem D, Warjri P. Antidiabetic, antioxidant, and TNF-α lowering properties of extract of the traditionally used plant *Ixeris gracilis* in alloxan-induced diabetic mice. *Pharmaceutical Biology*. 2015:53(4):494–502.
6. Dos-Santos HJF, Barbalho SM, Sgarbi JA. et al. The relationship between type 1 diabetes mellitus, TNF-α, and IL-10 gene expression. *Biomedicines.* 2023;11:1120.
7. Jang DI, Lee AH, Shin HY. The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. *International Journal of Molecular Sciences.* 2021;22:2719.
8. Reddy MK. Phenolic compounds in pomegranate (*Punica granatum* L.) and potential health bene–

fits. In Jayaprakasha GK, Patil BS, Gattuso G. Advances in plant phenolics: From chemistry to human health. 2018:201–223.

9. Hegazi NM, El-Shamy S, Fahmy H, Farag MA. Pomegranate juice as a super-food: A comprehensive review of its extraction, analysis, and quality assessment approaches. *Journal of Food Composition and Analysis*. 2021;97:103773.
10. Gimenez-Bastida JA, Avila-Galvez MA, Espin JC, Gonzales-Sarrias A. Evidence for health properties of pomegranate juices and extracts beyond nutrition: A critical systematic review of human studies. *Trends in Food Science and Technology.* 2021;114: 410-423.
11. Kandylis P, Kokkinomagoulos E. Food applications and potential health benefits of pomegranate and its derivatives. *Foods*. 2020;9(2):122.
12. Cheng J, Li J, Xiong RG, et al. Bioactive compounds and health benefits of pomegranate: An updated narrative review. *Food Bioscience*. 2023;53:102629.
13. Caruso A, Barbarossa A, Tassone A. Pomegranate: nutraceutical with promising benefits on human health. *Applied Sciences*. 2020;10(19):6915.
14. Singh J, Kaur HP, Verma A., et al. Pomegranate peel phytochemistry, pharmacological properties, methods of extraction, and its application: A comprehensive review. *ACS Omega.* 2023;8:35452-35469.
15. Neese F. Software update: The ORCA program system–version 5.0. *WIREs Computational Molecular Bioscience*. 2022;12(5):e1606.
16. Hsu KC, Chen YF, Lin SR, Yang JM. iGEMDOCK: A graphical environment of enhancing GEMDOCK using pharmacological interactions and post-screening analysis. *BMC Bioinformatics*. 2011;12 (Suppl.1):s33.
17. Arthur MN, Bebla K, Broni E. et al. Design of inhibitors that target the Menin-mixed-lineage leukemia interaction. *Computation.* 2024;12(1):3.
18. Bosque R, Maseras F. Performance of the semiempirical PM3 (tm) method in the geometry optimization of transition metal complexes. *Journal of Computational Chemistry.* 2000;21(7):562-571.