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IN SILICO STUDY OF APIGENIN AND ITS DERIVATIVES AS POTENTIAL INHIBITORS OF TNF-A AND MMP-9 FOR BURN WOUND HEALING

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ABSTRACT

The prevalence of burns ranks fourth in trauma after traffic accidents, falls, and violence. The prevalence of burns ranks fourth in trauma after traffic accidents, falls, and violence. The healing of burn wounds results from a complex inflammatory response where pro-inflammatory cytokines, for instance, TNF-α and MMP-9, play a pivotal role. Flavonoids with strong anti-inflammatory activity are apigenin, this compound comes from plants. In this study, we evaluated apigenin and its derivatives to determine their effectiveness as burn wound healing agents using molecular docking modeling techniques. To evaluate the molecular docking of these compounds with their target proteins, we used computer-aided drug design tools such as AutoDock Vina, PyMOL, and Discovery Studio. The data showed that apigenin compounds have better affinity than natural ligands to inhibit MMP-9 which can increase the rate of the inflammatory phase of burn wound healing. These findings indicate that apigenin and its derivatives have good potential to be candidates in the development of new therapeutic interventions for burn wound healing.

Keywords: Apigenin, burn, in-silico, MMP-9, TNF- α.

INTRODUCTION

The prevalence of burns ranks fourth in trauma after traffic accidents, falls, and violence. In fact, burns are one of the most common injuries worldwide (Greenhalgh, 2019). Due to a lack of preventive measures, burns have a significant impact on society due to their impact on health, which causes high mortality rates. In recent years, there has been an increase in the use of advanced wound healing techniques that can be applied clinically to address the increasing number of dermatological cases (Yang *et al.*, 2023). In less developed places such as

Indonesia, burns tend to cause greater severity due to inadequate medical facilities. The development of advanced wound care techniques, such as topical dressings and closure devices, tends to overshadow the need for tangential excision surgery (Lumintang *et al.*, 2021).

Burn injuries can be categorized based on their mechanisms, which include thermal, radiation, electrical, and chemical burns. Additionally, they are traditionally classified by severity into first, second, and third degrees (Slatter, 2003). The progression of burn wound pathogenesis occurs through three main phases: the inflammatory phase, characterized by the influx of immune cells such as neutrophils and macrophages; the proliferative or granulation phase, which encompasses myofibroblast formation, collagen synthesis, neovascularization, and epithelialization; and the remodeling phase, indicated by tissue regeneration. These phases are regulated by a variety of chemokines and cytokines of pro-inflammatory, including CSF-1, TNF-α, interleukin-1, and MCP-1, whose levels typically decrease as the wound-healing process advances (Rodero & Khosrotehrani, 2010).

Tumor necrosis factor alpha (TNF-α) plays an important role as a mediator of the inflammatory response by activating the immune system and increasing the expression of matrix metalloproteinase 9 (MMP-9). High levels of TNF-α can prolong the inflammatory phase, risk further tissue damage, and prevent the transition to the proliferation phase of the wound healing process. Research shows that the balance between TNF-α and MMP-9 significantly affects the speed and quality of wound healing, especially in burns, where excess TNF-α can slow the healing process (Marrassini *et al.*, 2020; Zhang *et al.*, 2015).

Apigenin has the ability to perform various broad biological activities such as antioxidant, neuroprotective, antiviral, anti-inflammatory and it shows low cytotoxicity to normal human cells. (Feng *et al.*, 2023). Apigenin can reduce inflammation by suppressing the production of pro-inflammatory cytokines and other inflammatory mediators (Charalabopoulos *et al.*, 2019). The results of research from Ma *et al.* (2022) showed that apigenin can reduce the levels of various pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β.

Rapid technological developments such as artificial intelligence, molecular docking and in silico studies, facilitate the use of effective natural compounds to be more efficient (Balachandran *et al.*, 2023). These developments are very important for

researchers to discover and develop new drugs by reducing interventions on experimental animals for preclinical testing.

There are still not many molecular docking studies on the use of active ingredients apigenin and its derivatives, especially as potential drugs for healing burns. Therefore, the current study aims to determine the apigenin compound and its derivatives that are effective against receptors that inhibit burn healing as a first step in the discovery of new drugs.

METHODS

Materials

This study was accomplished with a laptop computer featuring specifications of: Windows 10, 4 GB RAM, Intel Core i3 processor, and 64-bit x64 architecture with 4 computational tools. The AutoDock Tools 1.5.7 was used to perform docking simulations while molecular visualizations was achieved using PyMOL and advanced analyses were performed with Discovery Studio. Structural and Chemical data of the proteins and ligands used as targets were extracted from credible online resources such as the Protein Data Bank (PDB) and PubChem.

With respect to this study, the materials include Ligand and Protein Structures used in Molecular modeling. The Apigenin and its Derivatives structures were obtained from PubChem, while the TNF-α and MMP-9 protein receptors were obtained from the protein data bank (PDB). The above-mentioned structures are used as the basis for docking simulation and molecular interaction analysis.

Research Path

1. Protein Preparation and Preparation

In the first stage of the Procedure, it is necessary first to find the TNF-α and MMP-9 receptors since both these protein receptors are known to contribute to the inflammation as well as in the burn wound healing process (Costa *et al.,* 2024; Cabral-Pacheco *et al.,* 2020). The three-dimensional (3D) models of TNF-α and MMP-9 can be obtained from the Protein Data Bank (PDB) page (https://www.rcsb.org), with the corresponding PDB IDs 2AZ5 and 5UE4.

2. Ligand Preparation and Preparation

After the protein receptors were prepared, the next step, one needs to prepare test compounds, which combine nine active apigenin compounds and their derivatives and the natural ligands. The 3-D structures of apigenin derivatives and natural ligands were retrieved using the SDF data format, which is available on the PubChem database

(https://pubchem.ncbi.nlm.nih.gov). In addition, the ligands were created by adding Hydrogen using the Discovery Studio software, and the altered structures were exported into PDB format.

3. Validation of the Docking Simulation Approach

Molecular docking validation was conducted following the modifications of Sari *et al.* (2020) using Auto Dock Vina version 1.5.7. The natural ligand, 6,7-dimethyl-3-[(methyl{2-[methyl({1-[3-(trifluoromethyl)phenyl]-1H-indol-3-yl}methyl)amino]ethyl}amino)methyl]-4H-chromen-4-one, was redocked to the TNF-α receptor (PDB: 2AZ5), and N-[5-[2-[(2-methoxyphenyl)amino]-1,3-thiazol-4-yl]-4-methyl-1,3-thiazol-2-yl]ethanamide was redocked to the MMP-9 receptor (PDB: 5UE4). In selecting the optimal box for validation of molecular docking, the one that produced the lowest root mean square deviation (RMSD) and most closely matched the ligand conformation postdocking was selected.

4. Molecular Docking between Ligand Receptors

The analysis of the data was performed using AutoDock Vina. The 2D renders of the results were created in the Discovery Studio Visualizer, while focusing on the hydrogen bonds as well as the hydrophobic and amino acid residues that make interactions with the ligand and the protein. The 3D renders were obtained using PyMOL and were used to view the location of the ligands on the surface of the protein (Dallakyan & Olson, 2015).

Data Analysis

Molecular docking reveals hydrogen bond formation and binding energy. The mechanism of interaction is understood through hydrogen bond analysis, while the strength of the interaction between the ligand and the macromolecule is indicated by the binding energy. Stronger bond stability is associated with lower binding energy values.

RESULTS AND DISCUSSION

1. Validation of the Docking Simulation Approach

The proposed molecular docking procedure is accurate and reproducible, RMSD validation must take place. The reason assess the accuracy of the docking process is because measuring the structure of a native ligand before and after the docking experiment allows us to quantify conformational modifications in the system. In simple words, if the RMSD ratio is lower than 2 Å, the method is acceptable (Lohning *et al.*, 2017).

The parameters provided were utilized in the validation phase, and a valid docking procedure was defined as follows: the grid box for receptor TNF-alpha was set to center at x= -9.181, y=67.363, z=20.045 with a grid size of 40x 40 x 40 and spacing equal to 0.375. The same grid size and spacing were used for the MMP-9 receptor box set parameters x=48.145, y=64.14, z= 42.896. The docking procedure was considered valid, as redocking of the native ligand resulted in RMSD values below 2 Å, precisely 1.600 Å for TNF-α and 0.758 Å for MMP-9. These results confirm that molecular docking can be reliably performed on each protein using the same validated settings.

(a) **(b)**

Figure 1. Molecular docking validation results. (a) position of docking result ligand (blue) and natural ligand (green) on the TNF-α protein (b) the position of the position of the docking result ligand (green) and the (blue) on the MMP-9 protein

2. Molecular Docking between Ligands and Receptors

Molecular docking analysis of apigenin derivatives revealed potential interactions with two target proteins, TNF-α and MMP-9, as shown in Table 1. The nature of these interactions was evaluated based on binding energy (ΔG), the number of hydrogen bonds, and the specific amino acid residues involved.

Based on the obtained data, all test compounds exhibited negative binding energy values, indicating their potential as inhibitors of TNF-α and MMP-9 receptors. Among derivatives of apigenin, Vitexin exhibited the most favorable energy binding value with ΔG -8.3 kcal/mol which is in close proximity of the native ligand as a TNF-α inhibitor. Instead, Apigenin showed a higher binding potency of ΔG -8.9 kcal/ mol, which is indicative of stronger inhibition of MMP-9 than the native ligand. The assessment of potential therapeutic compounds is carried out through their binding affinity, which depend on the interactions of the receptor sides and the likeness of the binding pockets. (Patil *et al.*, 2010). The assessment of the change in free

energy of binding relates to the spontaneity of the reaction and the stability of the ligand-receptor complex. Lower values of ΔG suggest a stronger and more stable binding; therefore, a greater potential for the compound to interact with the biological target (Adelina, 2014).

Table 1. Results of ligand docking with the TNF-α and MMP-9 receptors using Autodock Vina.

No	Compound name	TNF-α			MMP-9		
		ΔG (kcal/ mol)	**Hydrogen bonds**	**Distances**	**ΔG (kcal/ mol)**	**Hydrogen bonds**	**Distances**
1	Apigenin 7-glucuronide	-8.2	SER D60 TYR151 GLY121	3.06Å 2.23Å 2.13Å	-8.2	ARG B106	3.35Å
2	Acacetin	-7.4	TYR151	2.44Å	-8.1	GLY B233	2.30Å
3	Apigenin trimethyl ether	7.3	SER D60 TYR D151	3.53Å 3.77Å	-7.9	GLY B233	3.33Å
4	Apigenin	-7.4	SER D60 TYR D151 TYRC151	2.46Å 3.16Å 2.29Å	-8.9	ARG B98	2.68Å
5	Apigetrin	-8.1	SER D60 TYR D151 TYRC151	1.95Å 2.18Å 3.08Å	-8.5	ASP B235 HIS B230	2.16Å 2.80Å
6	Genkwanin	-7.4	-	-	-8.1	GLY B233	2.45Å
7	Hispidulin	-7.4	SER D60	3.60Å	-8.2	ASP B235	3.56Å
8	Isovitexin	-8.0	SER D60 TYR D151 GLY C121	2.88Å 2.84Å 3.19Å	-8.7	ASP B113	1.93Å
9	Vitexin	-8.3	GYL C121 LEU C120 SER C60 TYR C151 GLN C61	2.29Å 2.64Å 2.29Å 3.06Å 2.02Å	-7.4	GLY B233	2.54Å
10	Native ligand	-8.6	LEU C120	3.53Å	-7.4	ASP B235	2.85Å

The hydrogen bonds formed by the Vitexin compound with TNF-α (Figure 2 and Table 1) include interactions with GYL C121, LEU C120, SER C60, TYR C151, and GLN C61, with bond distances ranging

from 2.02 to 3.06 Å according to He *et al.* (2005) TNF-α has active sites on amino acids L57, Y59, S60 Y119, L120, G121, and Y151 which show that almost all of them interact with vitexin. In comparison, the hydrogen bond formed between the natural ligand and TNF-α occurs at LEU C120 with a distance of 3.53 Å. For Apigenin, hydrogen bonds are formed with ARG B98 on MMP-9, with a bond distance of 2.68 Å

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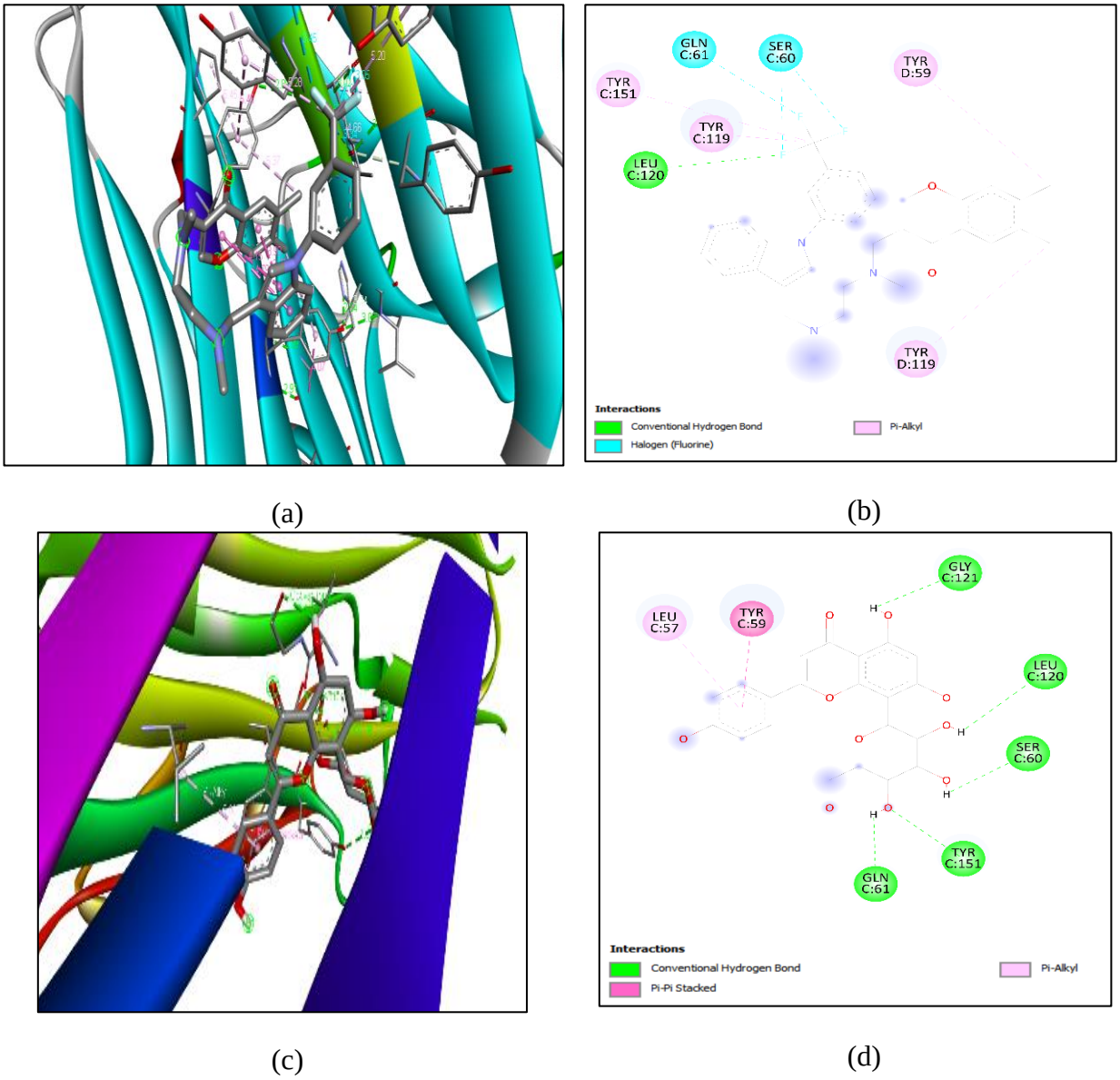

Figure 2. In silico visualization bound to TNF-α. (a) 3D visualization of Native Ligand Bound to TNF-α. (b) 2D visualization of Native Ligand Bound to TNF-α. (c) 3D visualization of Vitexin Bound to TNF-α. (d) 2D visualization of Vitexin Bound to TNF-α

In contrast, the hydrogen bond between the natural ligand and MMP-9 (Figure 3 and Table 1) is formed at ASP B235 with a distance of 2.85 Å. These results indicate that the hydrogen bonds between the test ligands and TNF-α or MMP-9 are more substantial than those between the natural ligands and the respective receptors (Trott & Olson, 2009).

TNF-α and MMP-9 play crucial roles in inflammation and burn wound healing. TNF-α is a cytokine that triggers the inflammatory response, promoting inflammation necessary to combat infections; however, excessive levels can lead to tissue damage, which hinders the healing process (Raziyeva *et al.,* 2021). MMP-9, a protein produced by inflammatory cells such as neutrophils, degrades extracellular matrix components during issue remodeling. However, uncontrolled MMP-9 activity can interfere with the formation of new tissue and delay wound healing (Lee & Kim, 2022).

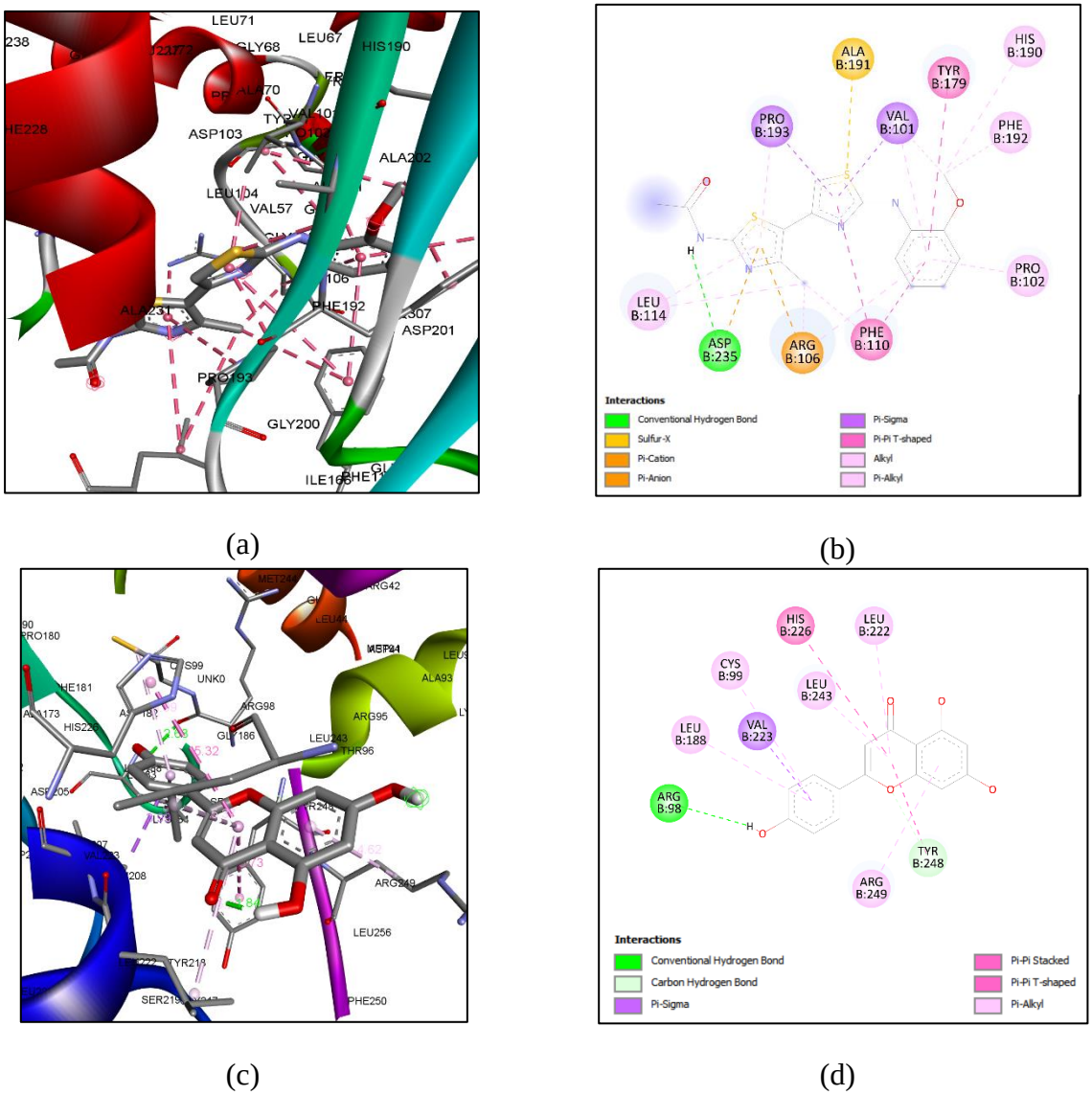

Figure 3. In silico visualization bound to MMP-9. (a) 3D visualization of Native Ligand Bound to MMP-9. (b) 2D visualization of Native Ligand Bound to MMP-9. (c) 3D visualization of Apigenin Bound to MMP-9. (d) 2D visualization of Apigenin Bound to MMP-9.

The results of the study by Noh *et al.* (2010) showed that in mice tumours induced by phorbol-12-myristate-13-acetate after administration of apigenin can inhibit the invasion and migration ability of Caski cells by reducing MMP-9 expression through suppression of the p38 MAPK signalling pathway. Meanwhile, in the study by Yang *et al.* (2019) in osteoarthritis patients, vitexin reduced the levels of pro-inflammatory cytokines IL-6, TNF-α, MMP-1, MMP-3 and MMP-13 in IL-1β-stimulated chondrocytes. The inhibition of TNF-α and MMP-9 expression is achieved through the use of specialized Apigenin derivatives, which makes it possible to

drastically reduce inflammation, protect the extracellular matrix, and accelerate the healing of burn wounds. Apigenin and its derivatives offer a remarkable therapeutic prospect for improving the efficacy of burn wound healing because they are capable of acting as dual inhibitors to these proteins.

CONCLUSION

The results of in silico studies in this study conducted using molecular docking simulations showed vitexin compounds from apigenin derivatives showed strong binding affinity to TNF-α which was comparable to the native ligand while the MMP-9 receptor showed good potential for apigenin which had lower affinity energy compared to other derivatives and its natural ligand. Vitexin to TNF-α receptor and apigenin to MMP-9 receptor have therapeutic potential for burn wound healing. In vitro and in vivo studies are needed to validate the results of the computational predictions and assess their clinical relevance.

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