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Kayısı Çekirdeğindeki Fitosterollerin Kolesterol Üzerine Etkisi-Moleküler Docking
Effect of Phytosterols in Apricot Kernel on Cholesterol-Molecular Docking

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ÖZET

Amaç: Moleküler Docking tekniklerini kullanarak kayısı çekirdeğindeki Beta-Sitosterol, Kampesterol ve Stigmasterolün kolesterol metabolizmasıyla ilişkili anahtar protein reseptörleriyle olan etkileşimlerinin lipid düşürücü etkilerini analiz etmektir.

Materyal ve Metot: Moleküler Docking analizleri Chimera 1.17.3, AutoDock Vina ve Discovery Studio yazılımları kullanılarak gerçekleştirildi. Hedef proteinlerin yapısal bilgileri (PDB ID'leri: 6UOX, 1N7D, vb.) RCSB protein veri bankasından elde edildi. Ligandlar sterik engeller kaldırılarak, eksik hidrojenler eklenerek, kısmi yükler atanarak ve enerji minimizasyonu gerçekleştirilerek docking için hazırlandı. Fitosterollerin hedef proteinlerle bağlanma afiniteleri hesaplanarak görselleştirildi.

Bulgular: Moleküler Docking sonuçlarına göre en yüksek bağlanma enerjileri fitosteroller ile 6UOX ve 1N7D reseptörleri arasında gözlendi. Beta-sitosterol, Kampesterol ve Stigmasterolün 6UOX reseptörü ile bağlanma enerjileri sırasıyla -8.7, -9.0 ve -9.5 kcal/mol, 1N7D reseptörü için bu değerler -9.2, -9.3 ve -9.5 kcal/mol hesaplandı. Yapılan bu analiz ligandlar ve reseptörlerin amino asitleri arasındaki önemli hidrojen bağlarını ve hidrofobik etkileşimlerinin önemli olduğunu vurgulamıştır.

Tartışma ve Sonuç: Elde edilen bulgular hem hidrojen bağlarının hem de hidrofobik etkileşimlerin fitosterollerin lipid düşürücü ajanlar olarak etkinliği için çok önemli olduğunu göstermektedir. Stigmasterol en yüksek bağlanma afinitesini sergilemiş olup LDL kolesterolü düşürmede ve dolayısıyla kardiyovasküler hastalık riskini azaltmada daha etkili bir ajan olma potansiyelini ortaya koymuştur. Bu sonuçlar fitosterollerin kolesterol seviyelerini dengelemesini ve genel kardiyovasküler sağlığı iyileştirmeyi amaçlayan diyet müdahalelerinde kullanılmasını desteklemektedir. Ancak terapötik uygulamaları kapsamlı bir şekilde keşfetmek için daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Kayısı Çekirdeği, Fitosteroller, Beta-Sitosterol, Kampesterol, Stigmasterol, Kolesterol.

ABSTRACT

Objective: To analyse the lipid-lowering effects of apricot kernel Beta-Sitosterol, Campesterol and Stigmasterol interactions with key protein receptors associated with cholesterol metabolism using molecular docking techniques.

Materials and Methods: Molecular Docking analyses were performed using Chimera 1.17.3, AutoDock Vina and Discovery Studio software. Structural information of the target proteins (PDB IDs: 6UOX, 1N7D, etc.) was obtained from the RCSB protein data bank. Ligands were prepared for docking by removing steric hindrance, adding missing hydrogens, assigning partial charges and performing energy minimisation. The binding affinities of phytosterols with target proteins were calculated and visualised.

Results: According to the molecular docking results, the highest binding energies were observed between phytosterols and 6UOX and 1N7D receptors. The binding energies of beta-sitosterol, campesterol and stigmasterol with the 6UOX receptor were -8.7, -9.0 and -9.5 kcal/mol, respectively, while these values for the 1N7D receptor were -9.2, -9.3 and -9.5 kcal/mol, respectively. This analysis emphasised the important hydrogen bonds and hydrophobic interactions between ligands and amino acids of receptors.

Discussion and Conclusion: The obtained findings indicate that both hydrogen bonds and hydrophobic interactions are crucial for the activity of phytosterols as lipid-lowering agents. Stigmasterol exhibited the highest binding affinity, demonstrating its potential to be a more effective agent in lowering LDL cholesterol and thus reducing the risk of cardiovascular disease. These results support the use of phytosterols in dietary interventions aimed at stabilising cholesterol levels and improving overall cardiovascular health. However, further research is required to comprehensively explore therapeutic applications.

Keywords: Apricot Kernels, Phytosterols, Beta-Sitosterol, Campesterol, Stigmasterol, Cholesterol.

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INTRODUCTION

High levels of cholesterol, especially low-density lipoprotein (LDL) cholesterol, which plays an important role in the structure of the body's cell membranes, hormone production and digestion of fats, have a strong association with cardiovascular diseases (CVD) (1). In contrast to this negative effect of LDL cholesterol, high-density lipoprotein (HDL) cholesterol reduces cardiovascular risks by removing excess cholesterol from the arteries. High LDL levels are also associated with diseases such as hypertension and chronic kidney disease. High cholesterol levels are also more common in individuals with diabetes and increase the harmful effects of diabetes on the vascular system (2). Another important effect of high cholesterol is its association with metabolic syndrome, which includes a series of conditions including obesity, insulin resistance and inflammation. Metabolic syndrome increases the risk of CVD and type 2 diabetes.

Management of cholesterol levels can be achieved by diet, lifestyle changes and pharmacological treatments when necessary to reduce the risk of disease. Herbal treatment in cholesterol management aims to balance cholesterol levels with natural components as an alternative to pharmacological treatment (3). Especially foods rich in fibre, omega-3 fatty acids, phytosterols and some antioxidants stand out in herbal therapies. Apricot kernel may be effective in reducing the risk of cardiovascular disease by helping to lower bad cholesterol (LDL) thanks to its rich phytosterols (4). Beta-sitosterol, Campesterol and Stigmasterol are among the phytosterol compounds found in the highest amount in apricot kernel.

Beta-sitosterol is the phytosterol compound found at the highest rate in apricot kernel. Beta-sitosterol plays an effective role in lowering LDL cholesterol levels by reducing cholesterol absorption. It also has anti-inflammatory and immune supportive properties (5). Campesterol; Although not as high as beta-sitosterol, it is abundant in apricot kernel. Campesterol has the potential to reduce LDL cholesterol levels and improve cardiovascular health (6). Stigmasterol; It is another important phytosterol found in apricot kernel. Stigmasterol regulates cholesterol metabolism and helps keep cholesterol levels in the body under control (7). These phytosterols form the basis of the cholesterol-lowering properties of apricot kernels and contribute to heart health together with healthy fatty acids. How these phytosterols interact with cholesterol has not been fully determined. Today, there are many molecular modelling techniques to understand how compounds interact with biological targets, their binding sites and mechanisms. Among these modelling techniques, molecular dynamics simulations, quantum mechanical calculations and docking methods are prominent.

Molecular docking calculations are often used to support experimental studies and to identify the active sites of molecules. Molecular modeling is an important method to study the interactions of molecules with proteins, and these calculations play a critical role in understanding the inhibitory mechanisms of proteins. Increased protein-molecule interactions are known to lead to an increase in the biological activity of molecules. These interactions are usually mediated by chemical forces such as hydrogen bonds, polar and hydrophobic interactions, π-π stacking and halogen bonding. Thus, it is the nature and intensity of these chemical interactions between the molecule and the protein that determine molecular activities. As the interactions between molecule and protein increase, the biological activities of molecules increase in parallel (8, 9).

In this study, we investigated the activity of Beta-Sitosterol, Campesterol and Stigmasterol molecules against PDB ID: 6OBU (PP1 Y134K in complex with Microcystin LR, Method X-RAY Dıffractıon, Resolution: 1.95 Å), PDB ID: 5KTF (Structure of the C-terminal transmembrane domain of scavenger receptor BI (SR-BI), PDB ID: 6UOX (Structure of itraconazole-bound NPC1, Method: Electron Mıcroscopy, Resolution: 4.02 Å), PDB ID: 1LE2 (Structural Basıs For Altered Functıon ın the Common Mutants of Human Apolıpoproteın-E, Method: X-Ray Dıffractıon Resolution: 3.00 Å), PDB ID:1N7D (Extracellular domain of the LDL receptor, Method: X-Ray Dıffractıon, Resolution: 3.70 Å) proteins were investigated (10).

MATERIALS AND METHODS

Molecular Docking

Structural information of target proteins for molecular docking studies were downloaded from https://www.rcsb.org. Molecular docking analyses were performed using Chimera 1.17.3, AutoDock Vina and Discovery Studio software. The structure of Beta-sitosterol, Campesterol and Stigmasterol molecule preferred as ligand was obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Proteins and ligand were prepared for molecular docking using the prepare a molecule for use as USCF input module in Chimera 1.17.3 program. In the preparation steps of molecular docking, steric hindrance was removed, missing hydrogens were added, partial charges were assigned, side chains were formed and missing loops were filled, water molecules located beyond 5 Å from the binding site in the crystal structures were removed, optimal energy minimization of the ligand was performed, potential binding sites of the proteins were determined and the grid box was adjusted. BIOVIA Discovery Studio 2021 Client (BIOVIA, San Diego, CA, USA) was used for visualization of 2D and 3D clamped poses (11).

RESULTS

Molecular modeling is an important method that uses molecular docking calculations to explain the structural and dynamical properties of biomolecular systems. Widely used in drug discovery and biotechnology, this method is of great importance in studying the interactions of molecules with proteins and the effects of these interactions on biological systems. Molecular docking analyzes how small molecules (ligands) bind to the active sites of target proteins and the energy levels of this binding. The docking score values of molecule-protein interactions are given in Table 1.

Table 1. Docking score values of molecule-protein interactions

		Docking score (kcol/mol)
6OBU	Beta-sitosterol	-7.6
	Campesterol	-7.2
	Stigmasterol	-7.4
5KTF	Beta-sitosterol	-7.5
	Campesterol	-7.6
	Stigmasterol	-7.8
6UOX	**Beta-sitosterol**	**-8.7**
	Campesterol	**-9.0**
	Stigmasterol	**-9.5**
1LE2	Beta-sitosterol	-7.1
	Campesterol	-7.3
	Stigmasterol	-7.0
1N7D	**Beta-sitosterol**	**-9.2**
	Campesterol	**-9.3**
	Stigmasterol	**-9.5**

The highest binding energies were between 6UOX and 1N7D receptors. The binding energies of Beta-sitosterol, Campesterol and Stigmasterol ligands with 6UOX receptor were -8.7, -9.0 and -9.5 kcal/mol, respectively, while these values were -9.2, -9.3 and -9.5 kcal/mol for 17ND, respectively.

It is known that proteins are inhibited due to the interaction of molecules with proteins. These interactions are usually chemical interactions and include hydrogen bonds, polar and hydrophobic interactions, π-π and halogen. In this model, the most important factor determining activity is the interaction between the molecule and the protein. The interaction between molecules and proteins is given in Figure 1.

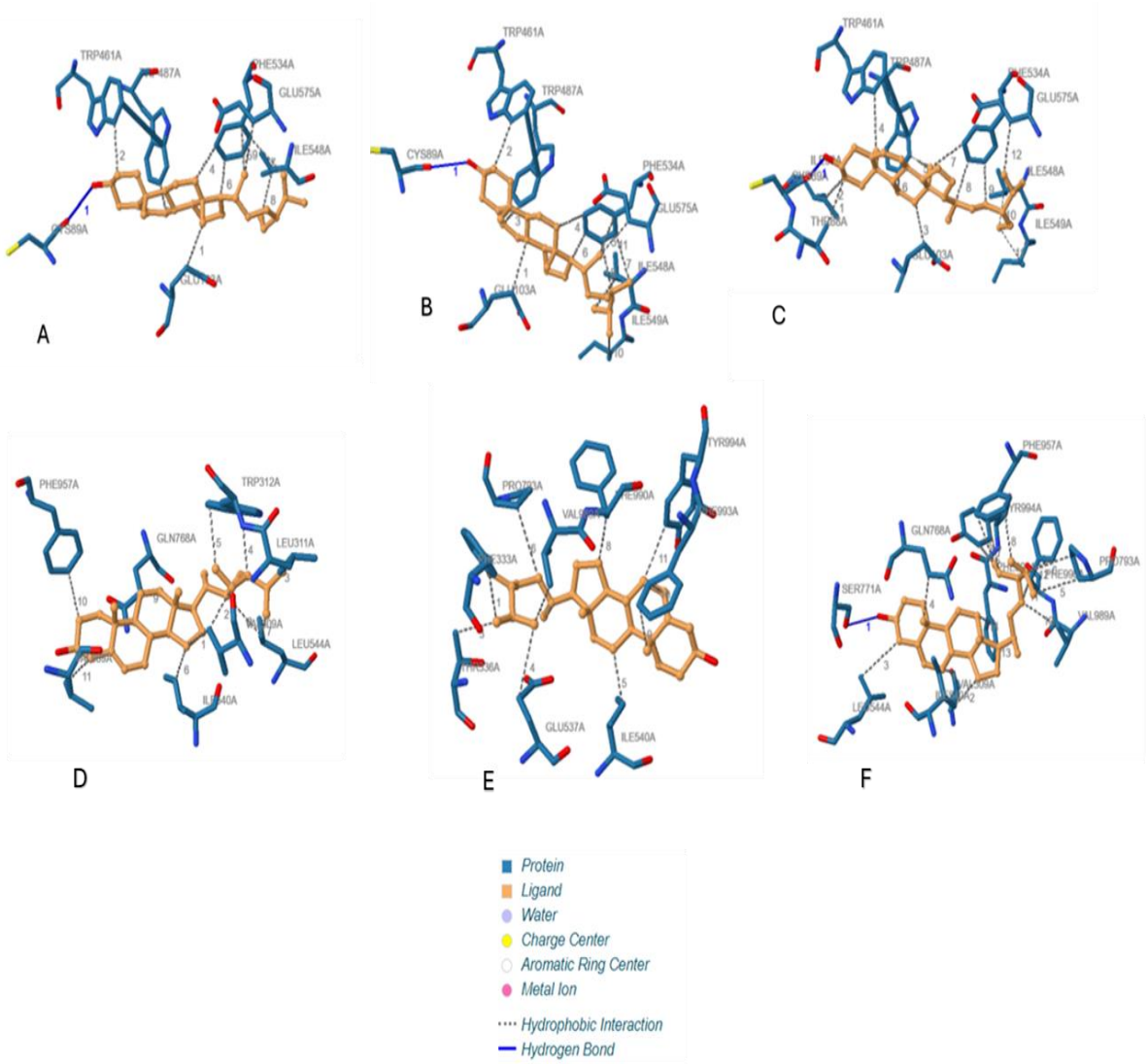

Figure 1. 3D structure of molecule-protein interactions (A: Beta-sitosterol-1N7D B: Campesterol-1N7D C: Stigmasterol-1N7D D: Beta-sitosterol-6UOX E: Campesterol-6U F: Stigmasterol-6UOX)

H bonds and hydrophobic interactions between ligands and receptors are given in Tables 2 and 3.

Table 2. H bonds and Hydrophobic interactions between Beta-Sitosterol, Campesterol and Stigmasterol and 1N7D

Hydrophobic Interactions					
Beta-Sitosterol		Campesterol		Stigmasterol	
AA	Distance	AA	Distance	AA	Distance
GLU	3.65	GLU	3.60	THR	3.43
TRP	3.51	TRP	3.50	ILE	3.68
TRP	3.61	TRP	3.57	GLU	3.58
PHE	3.93	PHE	3.85	TRP	3.82
PHE	3.70	PHE	3.75	TRP	3.29
PHE	3.36	PHE	3.38	TRP	3.68
PHE	3.69	PHE	3.44	PHE	3.30
ILE	3.75	PHE	3.66	PHE	3.38
GLU	3.58	ILE	3.84	PHE	3.39
		ILE	3.54	ILE	3.78
		GLU	3.27	ILE	3.41
				GLU	3.53
Hydrogen Bonds					
	AA	Distance H-A	Distance D-A	Donor Angle	
Beta-sitosterol	CYS	3.13	4.00	149.52	
Campesterol	CYS	2.95	3.74	138.68	
Stigmasterol	CYS	2.54	3.13	119.13	

Table 3. H bonds and Hydrophobic interactions between Beta-Sitosterol, Campesterol and Stigmasterol and 6UOX

Hydrophobic Interactions					
Beta-Sitosterol		Campesterol		Stigmasterol	
AA	Distance	AA	Distance	AA	Distance
VAL	3.46	PHE	3.33	VAL	3.13
VAL	3.03	PHE	3.62	ILE	3.14
LEU	3.14	THR	3.35	LEU	3.84
TRP	3.39	GLU	3.87	GLN	3.15
TRP	3.49	ILE	3.11	PRO	3.75
ILE	3.42	PRO	3.92	PRO	3.77
LEU	3.76	VAL	3.40	PHE	3.51
LEU	3.47	PHE	3.34	PHE	3.71
GLN	3.41	PHE	3.32	PHE	3.47
PHE	3.82	PHE	3.61	VAL	3.46
VAL	3.40	TYR	3.30	PHE	3.60
				PHE	3.80
				PHE	3.22
				PHE	3.59
				TYR	3.32
Hydrogen Bonds					
	AA	Distance H-A	Distance D-A	Donor Angle	
Stigmasterol	SER	2.26	3.16	153.54	

In 1N7D protein, H bonds were observed between Beta-sitosterol, Campesterol and Stigmasterol ligands and CYS amino acid. When the interactions are analyzed, we can say that hydrophobic interactions occur with PHE, ILE, GLU, TRP and GLU amino acids. In 6UOX

protein, H bonds were observed between stigmasterol ligands and SER amino acid. When the interactions are analyzed, we can say that hydrophobic interactions occur with VAL, LEU, PRO, GLN, PHE, ILE, GLU, TRP and GLU amino acids. In a study conducted by Zixing et al., it was reported that Sitosterol mainly interacts with His 434, Phe 234, Val 398, Arg 238 and Tyr 391 amino acids. Stigmasterol was mainly surrounded by the amino acid residues Phe 234, Val 398, and His 434 (12).

DISCUSSION

In order to better understand the activity of phytosterols in apricot kernel, which is the subject of our study, it is of great importance to examine the effects of compounds in relation to biologically active substances by molecular modelling. Determination of the effect of compounds by molecular modelling is a widely used method in chemistry, biology and pharmacology. Molecular modelling allows to study the three-dimensional structure and physicochemical properties of a molecule by computational and simulation techniques. This method is used to understand how compounds interact with biological targets, binding sites and mechanisms. Molecular modelling techniques include molecular dynamics simulations, quantum mechanical calculations and docking methods. With these techniques, the interaction potential and binding affinity of a compound with a target protein or enzyme are examined. It plays an important role especially in new drug discovery and understanding the effect of natural compounds with biological activity. Studying the effects of compounds associated with biologically active substances such as phytosterols by molecular modelling can help predict how these compounds work at the cellular level, which receptors they bind to and how they affect biochemical processes. This method accelerates research processes and reduces costs by predicting potential biological activity prior to laboratory testing.

Beta-sitosterol, Campesterol and Sigmasterol selected in our study are widely used as lipid-lowering agents. This phytosterols may reduce the risk of cardiovascular disease (CVD) by lowering low-density lipoprotein (LDL) cholesterol levels in the blood. Phytosterols such as Beta-sitosterol, Campesterol and Sigmasterol are homologues of cholesterol found in various fruits and vegetables and are present in the daily diet in a similar way to cholesterol (13, 14). The efficacy of these compounds as lipid-lowering agents is further supported by molecular modelling studies revealing their interactions with specific protein receptors.

In this study, the binding energies of these phytosterols with 6UOX and 1N7D receptors were investigated. The results showed that Stigmasterol had the highest binding energy with both

receptors (-9.5 kcal/mol). This suggests that Stigmasterol has a stronger affinity compared to the other two phytosterols and its lipid-lowering effect may be more pronounced.

Hydrogen bonds and hydrophobic interactions play an important role during the interaction of phytosterols with proteins. In 1N7D protein, hydrogen bonds were observed between beta-sitosterol, campesterol and stigmasterol ligands and CYS amino acid. Hydrophobic interactions with PHE, ILE, GLU and TRP amino acids were also detected. In 6UOX protein, hydrogen bonding between the stigmasterol ligand and SER amino acid and hydrophobic interactions with VAL, LEU, PRO, GLN, PHE, ILE, GLU, TRP and GLU amino acids were detected. These findings are also compatible with the results of the study by Zixing et al. (12). It was reported that sitosterol showed significant interactions with His 434, Phe 234, Val 398, Arg 238 and Tyr 391 amino acids and stigmasterol showed significant interactions with Phe 234, Val 398 and His 434 amino acids.

The findings of the present study indicate that both hydrogen bonding and hydrophobic interactions are vital in determining the efficacy of phytosterols as lipid-lowering agents. The structural diversity among different receptors and their respective interaction profiles can inform future drug design strategies aimed at enhancing the biological activity of these compounds. Understanding these molecular dynamics through advanced modeling approaches could contribute significantly to optimizing therapeutic interventions targeting cardiovascular diseases linked to cholesterol metabolism.

Herbal treatment may be preferable because it usually has fewer side effects and has a favourable effect on overall health in the long term. However, since its effects are generally slower and milder than pharmacological treatments, it may not be sufficient alone for high-risk individuals. Herbal treatment may be more effective, especially when used in combination with lifestyle changes and medical intervention when necessary.

CONCLUSION

According to the study, Beta-sitosterol, Campesterol and Stigmasterol had the highest binding energies with 6UOX and 1N7D receptors. According to the results obtained, Stigmasterol had the highest binding energy with both receptors. This indicates that Stigmasterol has a stronger affinity compared to the other two phytosterols and its lipid-lowering effect may be more pronounced.

Looking at future public health approaches, Beta-sitosterol, Campesterol and Stigmasterol found in apricot kernels could be used for the prevention of cardiovascular diseases. This article clearly emphasized that Beta-sitosterol, Campesterol and Stigmasterol have cardioprotective potential, but more detailed research is needed. Extensive research on the use

of phytosterols for preventive and therapeutic management of phytosterols should be conducted.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Ethics Approval and Consent

Ethics committee approval is not required for the study.

Conflict of Interest

The author(s) declare no potential conflicts of interest related to the research, authorship and/or publication of this article.

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None. This study has not been presented at any scientific event

Author Contributions

Tuğba Gül Dikme/Adem Necip/Reşat Dikme/Sinem Güneş: Article hypothesis, Molecular docking, Literature review, Writing.

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