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Molecular docking analysis of *Citrus aurantifolia* peel bioactive compounds against fshr, androgen receptor, and PCSK4 in hyperlipidemia-associated male reproductive dysfunction

Analisis molecular docking senyawa bioaktif kulit Citrus aurantifolia terhadap FSHR, reseptor androgen, dan PCSK4 pada gangguan reproduksi pria terkait hiperlipidemia

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Abstract

Hyperlipidemia is a diet-induced metabolic disorder that adversely affects systemic metabolism and reproductive function in males. Plant-derived bioactive compounds possess antioxidant, hypolipidemic, and metabolic regulatory activities. This study aimed to investigate the predicted interactions of bioactive compounds from C. aurantifolia peel with proteins involved in metabolic and reproductive pathways using an in silico molecular-docking approach. This in silico molecular docking study was conducted from January to April 2026 at the National Research and Innovation Agency in Indonesia. Ten bioactive compounds identified by GC–MS analysis of Citrus aurantifolia peel ethanol extract were used as test ligands FSHR (PDB ID: 2AM9), androgen receptor (PDB ID: 1XWD), and PCSK4 (UniProt ID: Q6UW60) were prepared using BIOVIA Discovery Studio 2021. Molecular docking was performed using AutoDock Vina in PyRx, with simvastatin as the reference ligand. The docking results were evaluated using the predicted binding affinities and interaction profiles. Pterin-6- carboxylic acid showed the most favorable predicted docking score for FSHR (−7.2 kcal/mol), whereas m-toluic acid showed the most favorable predicted docking scores for the androgen receptor and PCSK4 (−7.5 and −7.8 kcal/mol, respectively). In conclusion, the selected *C. aurantifolia* peel compounds showed predicted interactions with the three target proteins; however, molecular docking alone does not establish nutraceutical efficacy, bioavailability, safety, receptor activation, or clinical benefit. However, further experimental validation is required.

Keywords: Molecular docking, *Citrus aurantifolia*, hyperlipidemia, FSHR, androgen receptor, PCSK4

Abstrak

Hiperlipidemia merupakan gangguan metabolik yang dipicu oleh pola makan dan berdampak negatif terhadap metabolisme sistemik serta fungsi reproduksi pria. Senyawa bioaktif yang berasal dari tumbuhan memiliki sifat antioksidan, hipolipidemik, dan pengatur metabolisme. Penelitian ini bertujuan untuk menginvestigasi interaksi yang diprediksi antara senyawa bioaktif dari kulit *C. aurantifolia* dengan protein-protein yang berperan dalam jalur metabolik dan reproduksi menggunakan pendekatan *molecular docking* secara *in silico*. Penelitian *molecular docking in silico* ini dilaksanakan pada Januari hingga April 2026 di Badan Riset dan Inovasi Nasional (BRIN), Indonesia. Sebanyak 10 senyawa bioaktif yang diidentifikasi melalui analisis GC–MS terhadap

ekstrak etanol kulit *C. aurantifolia* digunakan sebagai ligan uji. Protein target yang digunakan meliputi FSHR (PDB ID: 2AM9), reseptor androgen (PDB ID: 1XWD), dan PCSK4 (UniProt ID: Q6UW60), yang dipreparasi menggunakan perangkat lunak BIOVIA Discovery Studio 2021. Proses *molecular docking* dilakukan menggunakan AutoDock Vina yang diimplementasikan dalam PyRx, dengan simvastatin sebagai ligan pembanding. Hasil *molecular docking* dievaluasi berdasarkan prediksi afinitas pengikatan (*predicted binding affinities*) dan profil interaksi molekuler. Pterin-6- carboxylic acid menunjukkan skor *docking* prediktif paling baik terhadap FSHR (−7,2 kcal/mol), sedangkan m-Toluic Acid menunjukkan skor *docking* prediktif paling baik terhadap reseptor androgen dan PCSK4, masing-masing sebesar −7,5 dan −7,8 kcal/mol. Sebagai kesimpulan, senyawa-senyawa terpilih dari kulit *C. aurantifolia* menunjukkan adanya interaksi yang diprediksi dengan ketiga protein target. Namun demikian, hasil *molecular docking* hanya memberikan prediksi komputasional dan belum dapat membuktikan efektivitas sebagai nutraseutikal, bioavailabilitas, keamanan, aktivasi reseptor, maupun manfaat klinis. Oleh karena itu, diperlukan validasi lebih lanjut melalui penelitian eksperimental untuk mengonfirmasi temuan tersebut.

Kata Kunci: Molecular docking, *Citrus aurantifolia*, hiperlipidemia, FSHR, reseptor androgen, PCSK4

Introduction

High-fat dietary patterns and the increasing consumption of energy-dense foods are major contributors to metabolic disorders, including obesity, dyslipidemia, and hyperlipidemia (Tareq et al., 2022). Lifestyle factors, such as dietary intake and physical activity, also influence cholesterol levels. Among the Prolanis participants, physical activity was identified as the strongest independent factor associated with cholesterol levels, although dietary factors showed an initial association (Nugroho et al., 2026).

Hyperlipidemia is characterized by elevated circulating lipid levels, which disrupt lipid homeostasis and promote oxidative stress. Excessive production of reactive oxygen species (ROS) may impair testicular function, alter hormonal regulation, and negatively affect spermatogenesis, ultimately contributing to male reproductive dysfunction (Khutami et al., 2022; Tareq et al., 2022). Beyond cardiovascular consequences, disturbances in lipid metabolism have increasingly been associated with impaired reproductive health through hormonal dysregulation, impaired spermatogenesis and oxidative damage (Funes et al., 2021; Mannucci et al., 2022; Zubi & Alfarisi, 2021). Consequently, nutritional interventions aimed at improving metabolic health have become an important area of research, particularly those involving food-derived bioactive compounds with antioxidant and hypolipidemic properties (Mehraban et al., 2021; Tanveer et al., 2017). Bioactive compounds, such as flavonoids and phenolic substances, may contribute to metabolic regulation by reducing oxidative stress and modulating pathways associated with lipid homeostasis (Chopra & Dhingra, 2021; Khutami et al., 2022).

Hyperlipidemia has emerged as a global health concern and has been increasingly associated with impaired male reproductive function through multiple biological mechanisms, including hormonal imbalance, oxidative stress, disruption of spermatogenesis, and altered sperm function (Zubi & Alfarisi, 2021). Infertility affects approximately one in six people of reproductive age worldwide and may result from male factors, female factors, a combination of both, or unexplained causes. Therefore, male reproductive disorders represent an important public health concern that requires further investigation (WHO, 2025). Lipids play crucial roles in maintaining membrane integrity, sperm viability, and the processes of maturation, capacitation, and fertilization. However, hyperlipidemic conditions induced by high-fat diets can disrupt lipid homeostasis, thereby negatively affecting male reproductive function (Zubi & Alfarisi, 2021).

At the molecular level, hypercholesterolemia disrupts the regulation of the Sterol Regulatory Element-Binding Protein 2 pathway in testicular

tissue, which plays a crucial role in lipid metabolism and spermatogenesis (Funes et al., 2021). Dysregulation of this pathway contributes to impaired sperm quality, including reduced motility and abnormal morphology (Oduwole et al., 2021). In addition, increased Reactive Oxygen Species (ROS) production may induce oxidative stress, leading to sperm cell damage and impaired male reproductive function (Mannucci et al., 2022). The primary therapeutic approach for hypercholesterolemia involves the use of lipid-lowering agents, particularly statins, which have been proven effective in reducing cholesterol levels and the risk of cardiovascular disease (Tanveer et al., 2017).

However, long-term statin use presents several limitations, including the risk of adverse effects and a relatively high rate of intolerance, which reportedly exceeds 30% (Al-Mohaissen et al., 2016; Tanveer et al., 2017). Previous studies have reported that statin exposure, particularly atorvastatin and simvastatin, may be associated with alterations in male reproductive parameters, including reduced sperm quality and abnormalities in sperm morphology, although the evidence remains inconclusive (Omolaoye et al., 2022). These findings support the investigation of complementary approaches to hypercholesterolemia management while carefully evaluating possible reproductive effects.

In recent decades, plant-based natural products have gained considerable attention as alternative therapeutic agents because of their broad biological activities and relatively favorable safety profiles (Khutami et al., 2022; Mehraban et al., 2021). Various phytochemicals present in medicinal plants exhibit antioxidant, anti-inflammatory, and hypolipidemic properties, which may help ameliorate metabolic disorders and protect reproductive function (Chopra & Dhingra, 2021). One such plant is *Citrus aurantifolia* (lime), which is rich in bioactive compounds, including flavonoids, limonene, pectin, and vitamin C (Czech et al., 2021). C. aurantifolia extracts have been reported to possess diverse pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial effects (Asmah et al., 2020; Galovicova et al., 2022; Indriyani & Susanto, 2024; Ouattara-Soro Fatou Shchérazade et al., 2021). Furthermore, previous studies have demonstrated that citrus extracts can improve sperm quality and quantity by reducing oxidative stress, particularly under toxic exposure conditions, such as cigarette smoke (Rahayu & Hanizar, 2022).

Citrus peels, often discarded as waste, contain substantial amounts of valuable bioactive compounds with potential health benefits. *C. aurantifolia* peel represents an underutilized food resource rich in flavonoids, phenolic compounds, limonoids, pectin, and vitamin C, which are recognized as dietary antioxidants and potential functional ingredients in nutraceutical formulations (Asmah et al., 2020; Czech et al., 2021). These bioactive compounds may contribute to metabolic regulation through antioxidant and hypolipidemic activities, thereby supporting the development of food-based therapeutic approaches for metabolic disorders (Khutami et al., 2022; Mehraban et al., 2021).

However, these studies primarily demonstrated antioxidant and hypolipidemic effects under experimental conditions and did not investigate the molecular interactions of individual bioactive compounds with reproductive proteins. Furthermore, in silico evidence regarding the interactions of *C. aurantifolia* peel bioactive compounds with FSHR, androgen receptor, and PCSK4 remains limited. Therefore, this study aimed to compare the predicted docking scores and molecular interaction profiles of selected *C. aurantifolia* peel compounds with simvastatin as the reference ligand against FSHR, androgen receptor, and PCSK4 using an in silico molecular docking approach. The findings provide computational predictions only, and further biological validation is required to confirm their relevance and therapeutic potential.

Methods

The molecular docking approach is widely used in drug discovery because of its cost-effectiveness and time efficiency in identifying potential drug candidates (El fadili et al., 2023). This in silico molecular docking study was conducted from January to April 2026 at the National Research and Innovation Agency (BRIN) in Indonesia. Protein and ligand preparation, molecular docking simulations, and molecular interaction analyses were performed by the research team in collaboration with BRIN

researchers using computational facilities and software available at BRIN.

The 10 compounds with the highest relative peak areas (%) were selected as test ligands from the 21 bioactive compounds previously identified via GC–MS analysis of the *C. aurantifolia* peel ethanol extract (Sitio et al., 2024). Molecular docking was performed according to the methodology described by Maulydia et al. (2025), which includes target preparation, ligand preparation, docking simulations, and analysis of docking results.

Simvastatin was used as the reference ligand because of its established role as a clinically approved lipid-lowering agent and its relevance as a pharmacological comparator in hypercholesterolemia-related studies. However, it should be noted that simvastatin is not a native or specific ligand for the target proteins analyzed in this study. The target proteins included Follicle-Stimulating Hormone receptor (FSHR; PDB ID: 2AM9), androgen receptor (PDB ID: 1XWD), and Proprotein Convertase Subtilisin/Kexin Type 4 (PCSK4; UniProt ID: Q6UW60). The data collection procedures involved ligand and protein preparation, active site determination, molecular docking simulation, and interaction analysis. Ligand structures were optimized before the docking analysis, and the protein structures were prepared by removing water molecules and unnecessary residues.

Data processing included docking simulations and visualization of two-dimensional (2D) and three-dimensional (3D) molecular interactions. Data analysis was performed descriptively based on the binding affinity values (kcal/mol) and interaction profiles, including hydrogen bonding and hydrophobic interactions. More negative docking scores were interpreted as more favorable predicted ligand–protein binding within the selected docking protocols. No inferential statistical analysis was conducted because this study used computational prediction methods (Maulydia et al., 2023, 2026; Rachmawati et al., 2022).

Ethical clearance was not required for this study, as it was conducted using computational methods and did not involve human participants, animal subjects, or identifiable personal data.

Result and Discussion

Molecular docking analysis was performed using ten selected bioactive compounds identified by GC–MS analysis of the *Citrus aurantifolia* peel ethanol extract. These compounds were selected based on their highest relative peak area (%) and used as test ligands. Simvastatin was employed as the reference ligand against three target proteins: The Follicle-Stimulating Hormone Receptor (FSHR; PDB ID: 2AM9), androgen receptor (PDB ID: 1XWD), and Proprotein Convertase Subtilisin/Kexin Type 4 (PCSK4; UniProt ID: Q6UW60). The three-dimensional structures of FSHR and androgen receptor were retrieved from the Protein Data Bank (PDB), whereas the PCSK4 protein sequence was obtained from the UniProt database for structural modeling prior to docking analysis.

Table 1. Docking-score results of bioactive compounds from ethanol extract of *C. aurantifolia* and simvastatin against FSHR, androgen receptor, and PCSK4

Compounds	Binding Affinity (kcal/mol)		
	FSHR	Androgen receptor	PCSK4
1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-	-5.6	-7.0	-6.6
2H-1-Benzopyran-2-one,7-methoxy-	-5.4	-6.4	-6.5
5-ethenyl-2-methoxyphenol	-4.9	-7.0	-5.9
6-methoxychroman-2-one	-5.4	-6.5	-6.5
Cis-vaccenic acid	-4.9	-6.7	-5.1
Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	-6.5	-6.2	-5.9
m-Toluic acid	-5.2	-7.5	-7.8
Neric_acid	-4.6	-6.5	-5.7
Pterin-6- carboxylic acid	-7.2	-6.5	-6.7
n-Hexadecanoic acid	-4.9	-6.7	-5.3
Simvastatin	-7.8	-8.2	-8.4

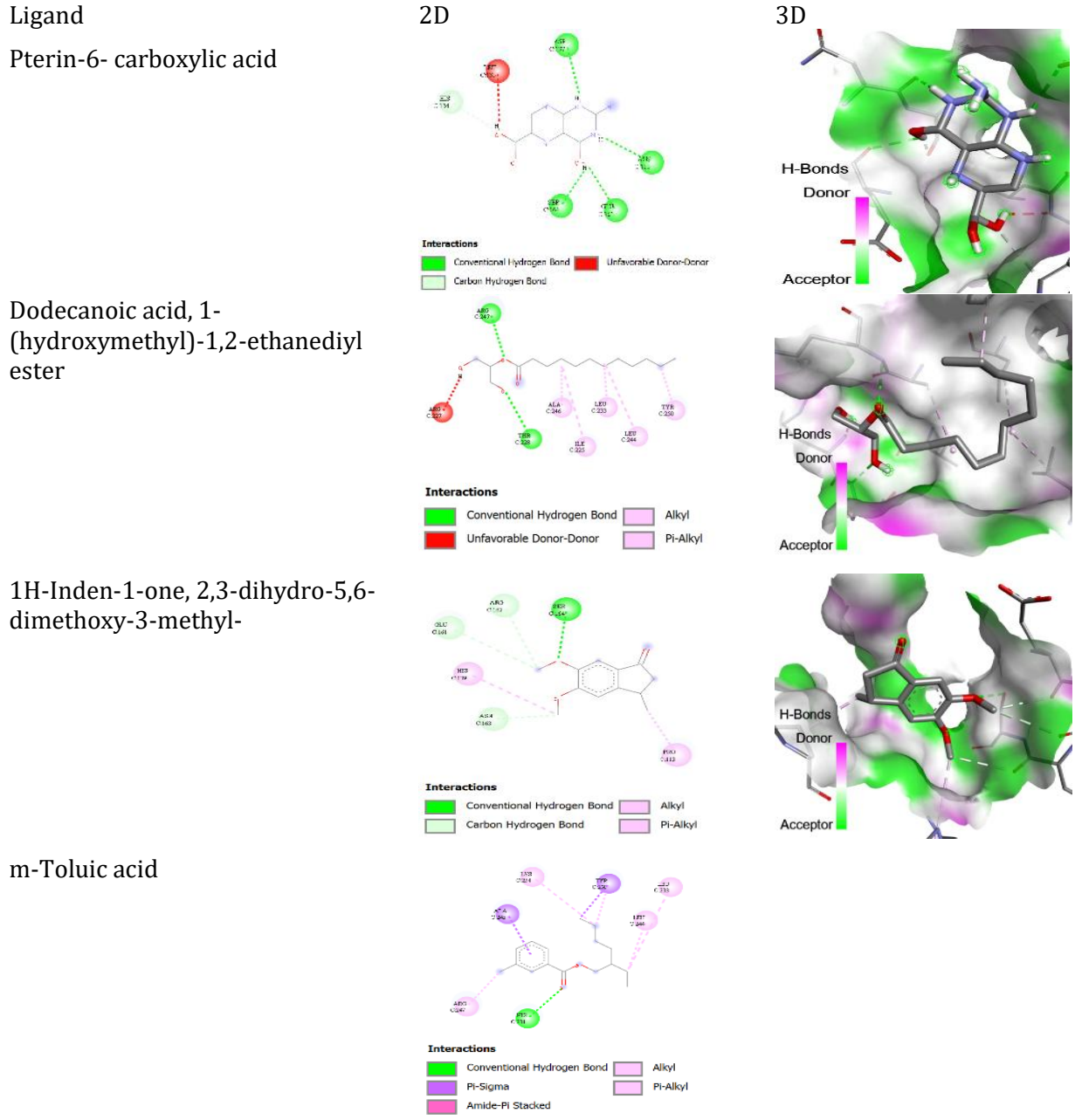
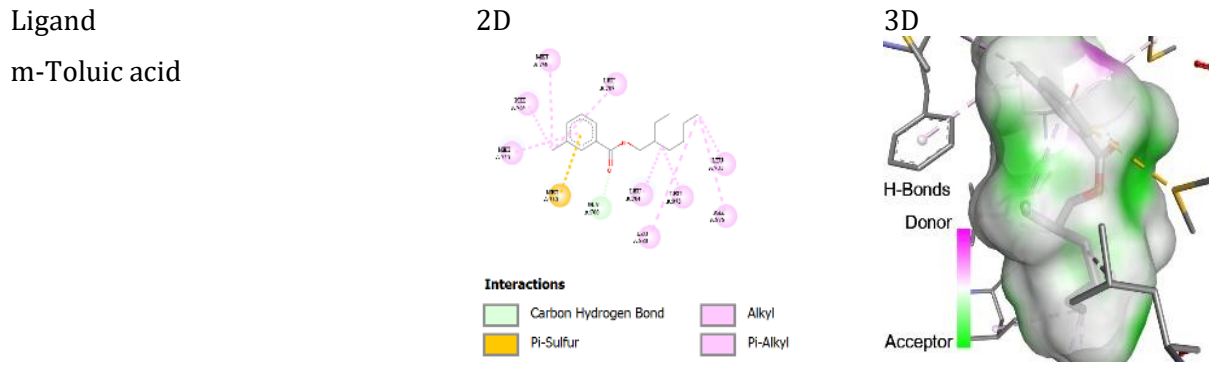
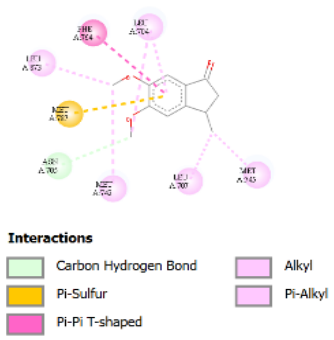
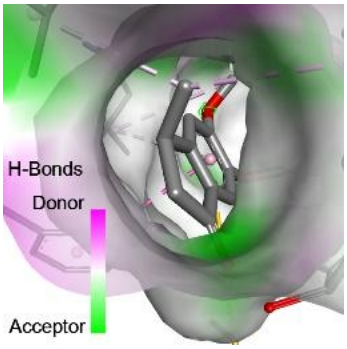


Figure 1. FSH receptor docking visualization (PDB ID: 2AM9) with Pterin-6- carboxylic acid, Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl esterand 1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl--

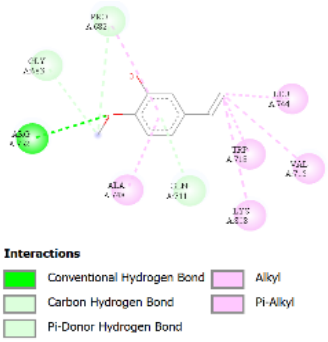


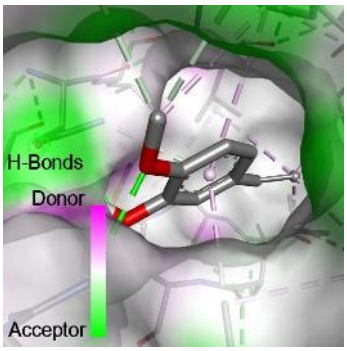
1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-



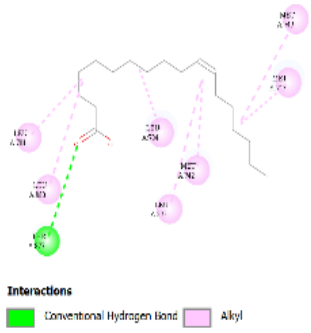


5-ethenyl-2-methoxyphenol





cis-vaccenic acid



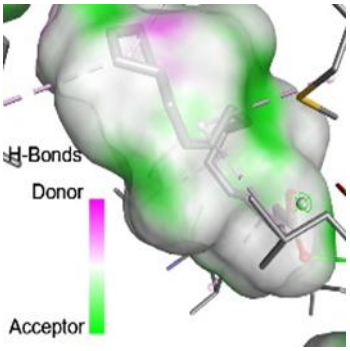
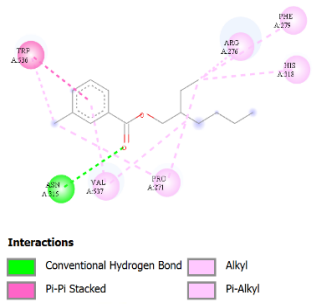
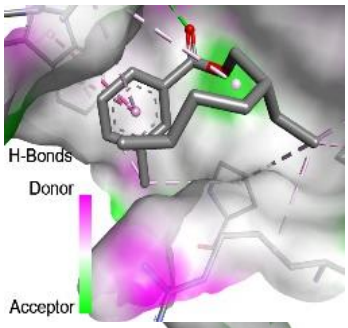
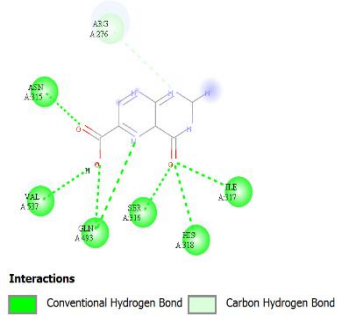
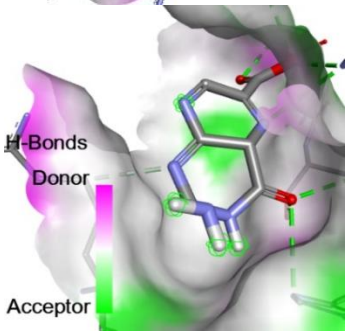


Figure 2. Androgen receptor docking visualization (PDB ID: 1XWD) with m-Toluic acid, 1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-, 5-ethenyl-2-methoxyphenol

Ligand	2D	3D
m-Toluic acid		
Pterin-6- carboxylic acid		

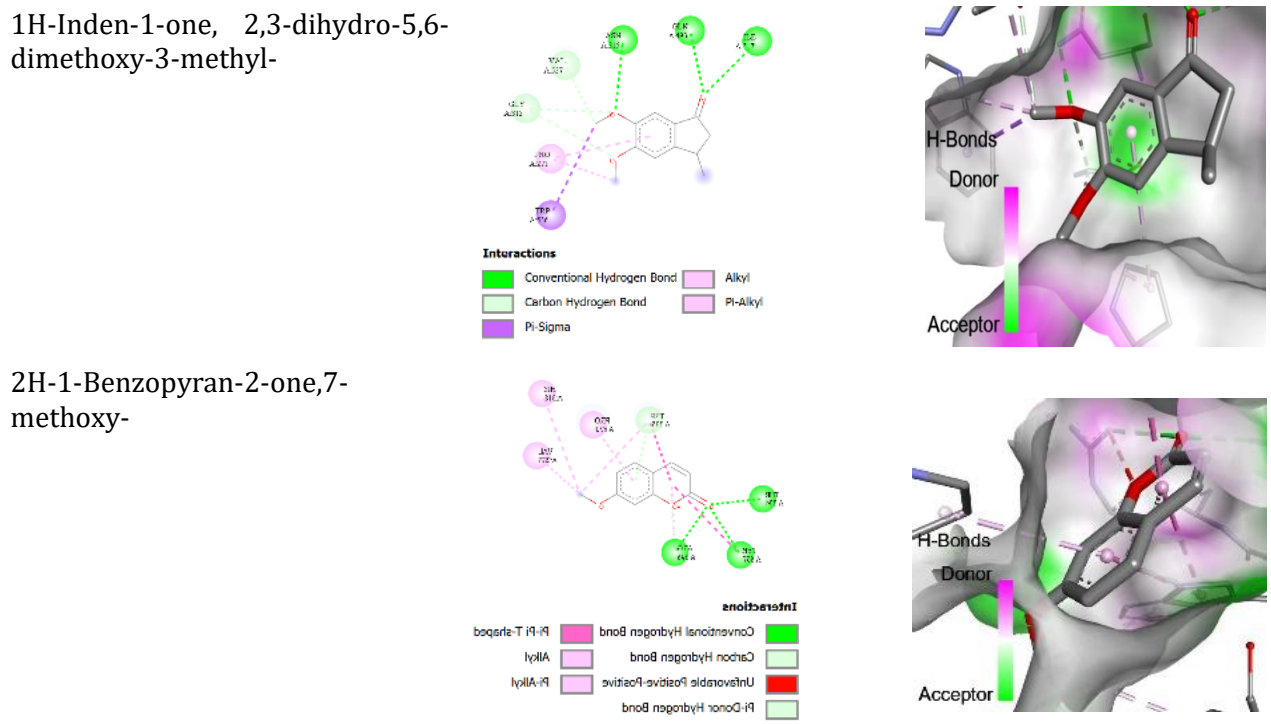


Figure 3. PCSK4 docking visualization (UniProt ID: Q6UW60) with m-Toluic acid, Pterin-6-carboxylic_acid, and 1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-

Molecular docking analysis was performed to evaluate the predicted binding interactions of the selected bioactive compounds from *C. aurantifolia* peel with three protein targets implicated in male reproductive function: follicle-stimulating hormone receptor (FSHR), androgen receptor (AR), and proprotein convertase subtilisin/kexin type 4 (PCSK4). Binding affinity values (kcal/mol) were used to evaluate the interaction strength, where more negative values indicated more favorable predicted binding within the selected docking protocol. Overall, the compounds produced docking poses with varying predicted scores for the three targets. Pterin-6-carboxylic acid exhibited the highest binding affinity toward FSHR (−7.2 kcal/mol), whereas m-toluic acid exhibited the strongest affinity toward the androgen receptor (−7.5 kcal/mol) and PCSK4 (−7.8 kcal/mol) (Table 1). As expected, simvastatin exhibited stronger binding to all targets. The molecular docking results provide only computational predictions of ligand–protein binding and do not establish biological activity, improved reproductive function, or clinical benefits.

Therefore, these findings should be regarded as preliminary computational evidence and require further validation through in vitro and in vivo studies. The amino acid residues from the molecular docking interactions are listed in Table 2. For FSHR, Pterin-6-carboxylic acid formed conventional hydrogen bonds with Asp137, Glu161, Asn163, and Ser164, a carbon–hydrogen bond with His134, and an unfavorable donor–donor interaction with Leu135; dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester engaged Thr228 and Arg247 through conventional hydrogen bonds while participating in alkyl and π-alkyl interactions with Leu233, Ala246, Ile225, Leu244, and Tyr250, alongside an unfavorable donor–donor interaction with Arg227; 1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl- formed a conventional hydrogen bond with Ser164, carbon–hydrogen bonds with Glu161, Arg162, and Asn163, and alkyl/π-alkyl contacts with Pro113 and His139; and m-toluic acid established a conventional hydrogen bond with His231, π-sigma and amide-pi stacked interactions with Ala246 and Tyr250, and alkyl/π-alkyl contacts with Leu233, Leu244, and Lys245.

For the androgen receptor, m-toluic acid formed a carbon–hydrogen bond with Gly708, a pi-sulfur interaction with Met745, and extensive alkyl and pi-alkyl contacts with Leu701, Leu704, Leu707, Met745, Phe746, Met749, Leu873, Phe876, and Leu880. 1H-Inden-1-one, 2,3-

dihydro-5,6-dimethoxy-3-methyl- engaged sn705 via a carbon–hydrogen bond, Met787 via a pi-sulfur interaction, Phe764 via a pi-pi T-shaped interaction, and Leu704, Leu707, Met742, Met745, and Leu873 through alkyl/pi-alkyl interactions. 5-ethenyl-2-methoxyphenol formed a conventional hydrogen bond with Arg752, a carbon–hydrogen bond with Gln711, pi-donor hydrogen bonds with Pro682 and Gly683, and alkyl/pi-alkyl interactions with Val715, Trp718, Leu744, Ala748, and Lys808. Cis-vaccenic acid engaged Thr877 via a conventional hydrogen bond and formed alkyl contacts with Leu701, Leu704, Met742, Met745, Leu880, and Leu873. For PCSK4, m-toluic acid formed a conventional hydrogen bond with Asn315, a stacked interaction with Trp536, and alkyl–alkyl contacts with Arg276, Pro271, Phe279, His318, and Val537. Pterin-6-carboxylic acid formed conventional hydrogen bonds with Asn315, Ser316, Ile317, His318, Gln493, and Val537, and a carbon–hydrogen bond with Arg. 1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-established conventional hydrogen bonds with Asn315, Ile317, and Gln493, carbon–hydrogen bonds with Hly312 and Val537, a interaction with Trp536, and an alkyl/-alkyl contact with Pro271. 2H-1-benzopyran-2-one, 7-methoxy- formed conventional hydrogen bonds with Thr394, Arg396, and Tyr657, carbon–hydrogen bonds with Trp395, T-shaped interactions with Trp395 and Tyr657, and alkyl/alkyl contacts with Val283, His318, and Pro672.

Collectively, these in silico predictions identified a range of hydrogen bonds, hydrophobic alkyl/pi-alkyl contacts, pi-stacking, and other non-covalent interactions that may contribute to the predicted binding of these compounds to their targets.

Table 2. Amino acid residues from the molecular docking study.

Receptors Target	Ligand	Amino Acid Residues
FSH	Pterin-6- carboxylic acid	Conventionnal Hydrogen Bond: Asp C:137, Glu C:161, Asn C:163, Ser C:164 Carbon Hydrogen Bond:His C:134 Unfavorable Donor-Donor: Leu C:135
	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	Conventional Hydrogen Bond: Thr C:228, Arg C:247 Alkyl and Pi-Alkyl: Leu C:233, Ala C:246, Ile C:225, Leu C:244, Tyr C:250 Unfavorable Donor-Donor: Arg C:227
	1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-	Conventional Hydrogen Bond: Ser C:164 Carbon Hydrogen Bond: Glu C:161, Arg C:162, Asn C:163 Alkyl and Pi-Alkyl: Pro C:113, His C:139
	m-Toluic acid	Conventionnal Hydrogen Bond: His C:231 Pi-Sigma and Amide-Pi Stacked: Ala C:246, Tyr C:250 Alkyl and Pi-Alkyl: Leu C: 233, Leu C:244, Lys C:245
Androgen receptor	m-Toluic acid	Carbon Hydrogen Bond: Gly A:708 Pi-Sulphur: Met A:745 Alkyl and Pi-Alkyl: Leu A:701, Leu A:704, Leu A:707, Met A:745, Phe A:746, Met A:749, Leu A:873, Phe A:876, Leu A:880
	1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-	Carbon Hydrogen Bond: Asn A:705 Pi-Sulphur: Met A:787 Pi-Pi T-Shaped: Phe A:764 Alkyl and Pi-Alklyl: Leu A:704, Leu A:707, Met A:742, Met A:745, Leu A:873
	5-ethenyl-2-methoxyphenol	Conventional Hydrogen Bond: Arg A:752 Carbon Hydrogen Bond: Gln A:711 Pi-Donor Hydrogen BondL Pro A:682, Gly A:683 Alkyl and Pi-Alkyl: Val A:715, Trp A:718, Leu A:744, Ala A:748, Lys A:808
	cis-vaccenic acid	Conventional Hydrogen Bons: Thr A:877

Receptors Target	Ligand	Amino Acid Residues
PCKS4	m-Toluic acid	Alkyl: Leu A:701, Leu A:704, Met A:742, Met A:745, Met A:745, Leu A:880, Leu A:873 Conventional Hydrogen Bond: Asn A:315 Pi-Pi Stacked: Trp A:536 Alkyl and Pi-Alkyl: Arg A:276, Pro A:271, Phe A:279, His A:318, Val A:537
	Pterin-6- carboxylic acid	Conventional Hydrogen Bond: Asn A:315, Ser A:316, Ile A:317, His A:318, Gln A:493, Val A:537 Carbon Hydrogen Bond: Arg
	1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-	Conevtional Hydrogen Bond: Asn A:315, Ile A:317, Gln A:493 Carbon Hydrogen Bond: Hly A:312, Val A:537 Pi-Sigma: Trp A:536 Alkyl and Pi-alkyl: Pro A:271
	2H-1-Benzopyran-2-one,7-methoxy-	Conventional Hydrogen Bond: Thr A:394, Arg A:396, Tyr A:657 Carbon Hydrogen Bond: Trp A:395 Pi-Pi T-Shaped: Trp A:395, Tyr A:657 Alkyl and Pi-Alkyl: Val A:283, His A:318, Pro A:672

The *in silico* docking data generated in this study suggest that certain bioactive compounds from *C. aurantifolia* peel may occupy the binding pockets of proteins implicated in male reproductive pathways relevant to lipid metabolism disorders (e.g., such as hyperlipidemia). These predicted binding energies primarily serve as computational filters to prioritize specific ligand–protein pairs for potential future investigations. Although lower (more negative) predicted binding energies are conventionally interpreted as indicative of stronger *in silico* binding affinity within a rigid static model, they do not prove physiological receptor–ligand interactions, nor do they reflect the actual stability of protein–ligand complexes in a dynamic cellular environment. Furthermore, the static nature of the docking algorithm employed here neglects protein flexibility, solvation effects, and entropic contributions, all of which critically govern real-world complex formation and stability. Crucially, these docking scores alone cannot establish, infer, or predict any biological activity, receptor agonism or antagonism, or therapeutic efficacy relevant to the male reproductive function or hyperlipidemia. This preliminary interpretation remains severely constrained by the complete absence of absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling and molecular dynamics (MD) simulations to assess conformational dynamics, as well as *in vitro* functional assays and pharmacological studies. Until such experimental data are obtained, these computational findings should be viewed solely as a theoretical and hypothesis-generating starting point, rather than as evidence of biological effects (Pinzi & Rastelli, 2019).

The docking scores indicate that the bioactive compounds of *C. aurantifolia* exhibit predicted binding interactions with target proteins involved in male reproduction. In molecular docking studies, more negative docking scores are generally associated with stronger predicted ligand–protein binding and may provide preliminary insights into potential molecular interactions (Alzain et al., 2024; Pinzi & Rastelli, 2019). However, as shown in Table 1, the reference control simvastatin consistently yielded more favorable (more negative) docking scores than all the tested C. aurantifolia compounds across the three target proteins, with values of −7.8, − 8.2, and − 8.4 kcal/mol for FSHR, androgen receptor, and PCSK4, respectively. Consequently, none of the bioactive compounds identified in this study should be interpreted as comparable, superior, competitive, or replacement agents for simvastatin based solely on these docking results. Instead, these computational findings provide only preliminary evidence that several *C. aurantifolia* compounds may be worthy of further investigation in future exploratory studies. Their potential biological activity and

therapeutic relevance remain speculative and require rigorous validation through ADMET analysis, molecular dynamics simulations, and comprehensive experimental investigations, including in vitro and in vivo studies. This cautious interpretation aligns with current research paradigms that emphasize natural products as valuable sources of novel chemical scaffolds for drug discovery rather than as direct substitutes for established, clinically proven therapeutic agents (Quintieri et al., 2024).

Molecular docking analysis predicted that the selected *C. aurantifolia* compounds interact with specific amino acid residues within the FSHR transmembrane allosteric binding pocket. These interactions primarily consisted of hydrogen bonds, hydrophobic interactions, and van der Waals contacts. However, it is critical to emphasize that molecular docking is a static predictive tool that only estimates the binding affinity and spatial complementarity. These computational predictions do not demonstrate receptor activation, induced conformational changes, G-protein coupling, or downstream signaling cascades. Moreover, no conclusions regarding enhanced Sertoli cell function or improved spermatogenesis can be drawn from these in silico results alone. Consequently, the predicted interactions with FSHR should be strictly interpreted as preliminary computational evidence of binding potential, rather than functional activity. Robust experimental validation, including competitive receptor-binding assays, cell-based functional studies (e.g., cAMP or β-arrestin recruitment assays), and comprehensive in vivo investigations, is urgently required to establish whether these ligands act as agonists, antagonists, or neutral binders and to determine their actual biological significance (Funes et al., 2021; Wang et al., 2022).

Ligand binding to the extracellular domain of the Follicle-Stimulating Hormone Receptor (FSHR) can trigger conformational changes in the transmembrane domain, which in turn activate G proteins and downstream signaling pathways (Wang et al., 2022). This indicates that interactions involving key residues, such as serine, tyrosine, and aspartate, are not only important for binding affinity but also play a critical role in biological signal transduction that determines cellular responses. Flavonoid compounds are known to interact strongly with target proteins through their hydroxyl (–OH) groups. These groups function as both hydrogen bond donors and acceptors, thereby enhancing the stability of the ligand–protein complexes. Such interactions may improve the binding affinity and specificity toward target receptors, including FSHR. These compounds can interact with proteins via their hydroxyl groups, increasing the likelihood of stable hydrogen bond formation (Chopra & Dhingra, 2021).

Molecular docking results on the androgen receptor indicated a predominance of hydrophobic interactions within the ligand-binding domain. This finding is consistent with the characteristic features of the androgen receptor, which possesses a specific hydrophobic pocket designed to accommodate endogenous ligands, such as testosterone. These hydrophobic interactions typically involve nonpolar residues, such as leucine, valine, and phenylalanine, which play crucial roles in stabilizing the ligand–receptor complex. In addition, van der Waals interactions further strengthen ligand binding within the active site of the receptor. Ligand binding to the ligand-binding domain of the androgen receptor induces conformational changes that enable receptor activation and subsequent translocation to the nucleus, where it regulates target gene expression. This pathway plays a critical role in spermatogenesis, particularly during spermatid maturation into functional spermatozoa (Matsumoto et al., 2018). (Therefore, compounds with a high affinity for the androgen receptor may act as modulators, either as partial agonists or antagonists, depending on the stability and nature of the interactions formed.

Androgen regulation plays a crucial role in maintaining hormonal balance in the male reproductive system. Disruptions in this pathway may lead to decreased sperm quality and quantity, ultimately resulting in male infertility (Oduwole et al., 2021). In this context, the ability of a ligand to establish stable interactions with the androgen receptor (AR) is an important parameter in molecular docking studies for identifying potential bioactive compounds. The bioactive compounds identified in *Citrus aurantifolia* peel belong to diverse chemical classes, including phenolic compounds, coumarin derivatives, fatty acids, aromatic carboxylic acids, and pteridine derivatives, among others. Depending on their chemical structures, these compounds may interact with the AR ligand-binding pocket through hydrogen bonding,

hydrophobic interactions, or π-interactions. Similar interaction patterns have been described in molecular docking studies of natural compounds targeting the androgen receptor, where hydrogen bonds, hydrophobic contacts, and π-interactions contribute to the predicted stability of ligand–receptor complexes (Fitri et al., 2026). PCSK4 is a protease that plays an important role in male reproduction by processing proteins involved in sperm capacitation, the acrosome reaction, and sperm–oocyte interaction. This enzyme is highly expressed in testicular tissue and is required for normal acrosomal function and successful fertilization (Dahril et al., 2019; Rose et al., 2021). Owing to its essential role in fertilization, PCSK4 has also been employed as a molecular target in recent in silico studies investigating reproductive bioactive compounds, including molecular docking analysis of natural compounds from *Piper nigrum* (Fitri et al., 2026). In the present study, several *Citrus aurantifolia*-derived compounds were predicted to interact with the PCSK4 binding site, suggesting their potential to bind to this reproductive target in bulls. However, molecular docking predicts only ligand–protein binding interactions and does not determine whether these interactions activate, inhibit, stabilize, or otherwise modulate PCSK4 function. Therefore, the predicted interactions should be regarded as preliminary computational evidence of ligand binding and require further validation through molecular dynamics simulations, in vitro functional assays, and in vivo studies before any conclusions regarding their biological or therapeutic significance can be drawn (Dahril et al., 2019; Rose et al., 2021).

Ligand-binding interactions involving active residues, such as histidine and aspartate, suggest the participation of catalytic mechanisms commonly observed in protease enzymes, where these amino acid residues play essential roles in peptide bond hydrolysis. The formation of hydrogen bonds and electrostatic interactions with these active residues may alter enzymatic function by acting as potential inhibitors or as modulators. In molecular docking analysis, a stronger binding affinity toward PCSK4 indicates that bioactive compounds may possess the ability to selectively regulate enzymatic activity, thereby potentially influencing the fertilization process. Furthermore, in addition to direct interactions with target proteins, the biological effects of compounds derived from *C. aurantifolia* may also be associated with their antioxidant activity. Flavonoids and phenolic compounds have been reported to suppress the generation of reactive oxygen species (ROS), which are major factors contributing to sperm damage under hyperlipidemic conditions (Mannucci et al., 2022).

Hyperlipidemia significantly affects the male reproductive system through various molecular mechanisms. This condition can disrupt androgen regulation by reducing testosterone levels and impairing testosterone signaling, both of which play essential roles in spermatogenesis. In addition, hyperlipidemia may impair the function of the Follicle-Stimulating Hormone Receptor (FSHR), thereby disrupting Sertoli cell activity, which is crucial for the development and maturation of spermatozoa. Furthermore, excessive lipid accumulation can increase the production of reactive oxygen species (ROS), leading to oxidative stress, which contributes to sperm DNA damage, reduced motility, and morphological abnormalities (Funes et al., 2021). Previous studies have reported that Citrus aurantifolia contains bioactive compounds with antioxidant properties (Sitio et al., 2024). However, the antioxidant activity was not evaluated in the present study. Therefore, the molecular docking results should be interpreted solely as preliminary computational predictions of ligand–protein binding interactions and not as evidence of antioxidant activity or biological efficacy of the compounds.

The bioactive compounds present in *C. aurantifolia*, particularly coumarins, phenolics, and fatty acids, may counteract the metabolic disturbances associated with hyperlipidemia through multiple biological mechanisms. Their antioxidant activity may reduce excessive *Reactive Oxygen Species* (ROS) production and protect reproductive cells from oxidative damage, thereby helping maintain physiological balance under conditions of metabolic stress. Furthermore, molecular docking results demonstrated potential modulatory effects on target proteins, such as the FSH receptor and androgen receptor, suggesting possible roles in hormonal regulation and reproductive function. In addition, the hypolipidemic properties of these compounds may contribute to improved lipid metabolism and reduced metabolic imbalance, thereby indirectly

creating a more favorable physiological environment for male reproductive health.

Beyond the predicted molecular interactions, our findings suggest that *Citrus aurantifolia* peel contains bioactive compounds that warrant further investigation for their potential nutritional and nutraceutical applications. However, the current molecular docking results alone are insufficient to support product development or health-related claims. Future studies should evaluate the standardized extract composition, optimal dosage, bio accessibility, bioavailability, food-matrix stability, ADMET properties, and toxicological profiles, along with experimental validation of biological efficacy through *in vitro* and *in vivo* studies. Only after these aspects have been comprehensively established can the feasibility of developing citrus peel–based dietary supplements, functional foods, fortified products, and other nutraceutical formulations be appropriately assessed. Therefore, the present findings should be regarded as preliminary computational evidence that may guide future research rather than support immediate nutritional or therapeutic applications (Durmus et al., 2024; Fekete et al., 2025; Granato et al., 2020; Santini et al., 2018).

Simvastatin was used as the reference ligand because of its well-established role in the management of hyperlipidemia (Bravo et al., 2022; Sitio et al., 2024). In the present study, all tested Citrus aurantifolia bioactive compounds exhibited less favorable docking scores than simvastatin across the three target proteins (FSHR, androgen receptor, and PCSK4). Nevertheless, the predicted ligand–protein interactions suggest that several compounds warrant further computational and experimental investigations to better understand their potential roles in metabolic and reproductive pathways. These findings should be regarded as preliminary computational evidence only and do not support claims of therapeutic efficacy or nutraceutical applications. Future studies incorporating ADMET analysis, molecular dynamics simulations, and in vitro and in vivo validation are required to determine the biological activity, safety, and nutritional relevance of these compounds.

Conclusion

Molecular docking analysis identified pterin-6-carboxylic acid and m-toluic acid as the leading test ligands among the bioactive compounds of *Citrus aurantifolia* peel, exhibiting the most favorable predicted docking scores for the evaluated target proteins. Specifically, pterin-6-carboxylic acid showed the strongest predicted binding to FSHR and PCSK4, whereas m-toluic acid demonstrated the most favorable predicted interaction with androgen receptors.

However, simvastatin exhibited more favorable docking scores than all the tested *Citrus aurantifolia* compounds across FSHR, androgen receptor, and PCSK4. Therefore, the present findings should be regarded as preliminary computational evidence of predicted ligand–protein interactions. Further studies, including ADMET analysis, molecular dynamics simulations, and experimental validation through *in vitro* and *in vivo* investigations, are required to determine the biological significance of these predicted interactions.

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