

Molecular Docking and Molecular Dynamics Analysis of Phytochemical Compounds from Nutmeg Fruit (*Myristica fragrans* Houtt.) on Androgen Receptor (4K7A) as Anti-Alopecia Therapy

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ABSTRACT

Androgenetic alopecia (AGA) represents the most prevalent hair loss form, necessitating natural treatment alternatives. This study investigated nutmeg (*Myristica fragrans* Houtt.) phytochemicals as anti-alopecia agents through molecular docking and dynamics simulations. Thirty-four compounds, including native ligands (minoxidil, finasteride) and 32 nutmeg-derived compounds, were evaluated against androgen receptor (PDB: 4K7A) using AutoDock4.0 and GROMACS 2024.2. Molecular docking validation achieved successful minoxidil redocking with 2.25 Å RMSD, confirming method reliability. Geranylgeraniol (ID: 33) demonstrated optimal results with -7.00 kcal/mol binding energy, nearly equivalent to finasteride (-7.19 kcal/mol) and superior to minoxidil (-4.85 kcal/mol). Geranylgeraniol formed strong hydrogen bonds with GLU793 at 1.77 Å distance, with nearby residues LEU862, TYR857, ARG854, LYS861, and LEU797. Twenty-five nanosecond molecular dynamics revealed stable complex formation with 0.23-0.25 nm RMSD values, indicating acceptable binding stability. RMSF analysis showed geranylgeraniol induced higher structural flexibility in specific regions, potentially enhancing biological activity. SASA analysis demonstrated lower surface area values (112.5 nm²), indicating improved stability versus minoxidil. These findings suggest geranylgeraniol possesses significant anti-alopecia potential with comparable finasteride efficacy and superior minoxidil performance.

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Introduction

Androgenetic alopecia (AGA), commonly known as male pattern baldness, represents the most prevalent form of progressive hair loss [1]. This condition results from genetic factors or androgen hormone activity, particularly dihydrotestosterone (DHT). DHT, an endogenous sex hormone, plays crucial roles in secondary hair growth in males but exhibits adverse effects on scalp hair follicles [2]. This hormone is produced through testosterone conversion by 5 α -reductase (5 α R) enzyme, comprising three isoenzymes: 5 α R1, 5 α R2, and 5 α R3. Isoenzymes 5 α R1 and 5 α R2 primarily contribute to AGA, with high activity in hair follicles, sebaceous glands, and genitourinary tract [3].

Elevated DHT levels in hair follicles cause follicular miniaturization, inhibiting anagen phase (growth phase) and accelerating

transition to catagen (transition phase) and telogen (resting phase) [4]. Consequently, hair follicles become inactive, shrink, and eventually cease producing new hair. Although AGA is not life-threatening, its psychological impact can be significant, including reduced quality of life and mental health issues.

Currently, FDA-approved AGA treatments include finasteride and minoxidil. Finasteride inhibits 5 α -reductase activity, while minoxidil acts as a vasodilator enhancing blood flow to hair follicles, stimulating hair growth [5]. However, both medications have long-term side effects, including skin irritation, itching, redness, and risks for pregnant and nursing mothers [4], [5], [6], [7]. Therefore, research into alternative treatments with low toxicity and minimal side effects becomes highly relevant.

Herbal plants have long served as traditional medicine sources for various

diseases, including alopecia. Several phytochemical compounds from herbal plants reportedly possess abilities to inhibit 5 α -reductase enzyme activity and reduce DHT levels, enabling their use as anti-alopecia agents such as *Verbascum xanthophoeniceum* [8], Saw palmetto (*Serenoa repens*), Green tea (*Camellia sinensis*), Pumpkin seed (*Curcubita pepo*), Rosemary (*Rosmarinus officinalis*), Grape seed (*Vitis vinifera*), and Licorice (*Glycyrrhiza glabra*) [9]. One intriguing plant for exploration is nutmeg (*Myristica fragrans* Houtt), known to contain various phytochemical compounds including monoterpenoids, sesquiterpenoids, phenolics, and alkaloids. These compounds possess potential as androgen receptor inhibitors and antioxidants, potentially reducing hair loss [10].

Nutmeg contains major bioactive compounds including myristicin, eugenol, elemicin, geranylgeraniol, and safrole that demonstrate potent antioxidant and anti-inflammatory activities [11]. Alopecia areata is a chronic inflammatory disease characterized by increased oxidative stress and reduced activity of the endogenous antioxidant system. This imbalance contributes to hair follicle damage and the progression of hair loss [12]. Antioxidant components such as quercetin have been proven to inhibit DHT production from testosterone by blocking α -5 reductase enzyme action and reducing oxidative stress, indicating that the antioxidant and anti-inflammatory constituents of nutmeg potentially address both major pathogenic factors of androgenetic alopecia through different yet complementary pathways.

This research aims to evaluate anti-alopecia activity of phytochemical compounds in nutmeg through in silico study approaches. The target protein used is androgen receptor with PDB code 4K7A, which was selected as it provides a validated structural template with minoxidil as co-crystallized ligand and has been successfully utilized in previous anti-alopecia studies for evaluating natural compounds [13]. The androgen receptor represents a primary target in alopecia pathogenesis through its interaction with dihydrotestosterone (DHT), which causes follicular miniaturization. Ligands used in docking include various

phytochemical compounds from nutmeg, such as (+)-4-Carene, γ -Terpinene, Linalool, Myristicin, and others [14], [15], [16]. Through molecular docking and molecular dynamics techniques, this study aims to identify compounds with high binding affinity to androgen receptors and stable binding interactions, potentially developed as topical medications for AGA treatment. These research findings are expected to provide scientific foundations for developing safer and more effective alternative therapies for androgenetic alopecia.

Materials and Methods

Hardware and Software

The hardware utilized in this research included a PC with Linux Ubuntu operating system, AMD Ryzen 9 9900X 12-Core 4.40 GHz processor, NVIDIA RTX 4080 Super graphics card, and 32 GB RAM. Analysis was conducted using Discovery Studio Visualizer, AutoDock 4, ADME-Tox pkCSM tools, and GROMACS 2024.2.

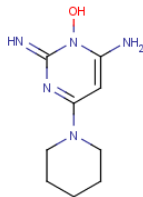
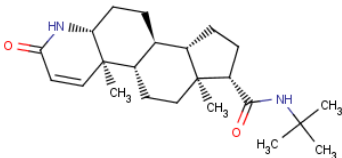
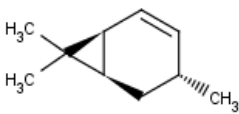
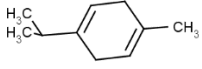
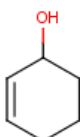
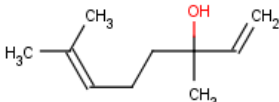
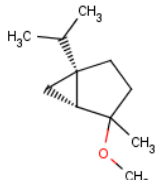
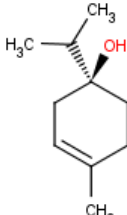
Materials

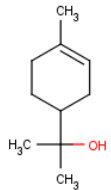
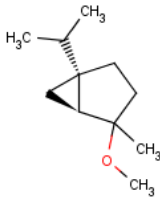
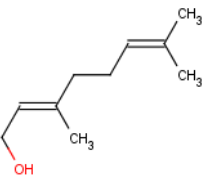
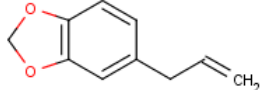
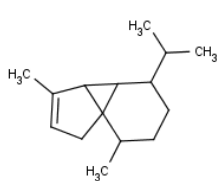
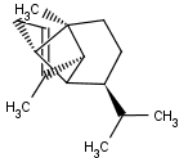
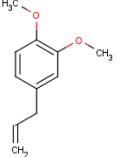
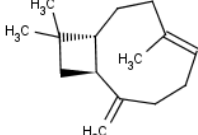
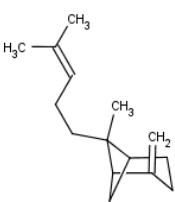
The crystal structure of androgen receptor (PDB code 4K7A) shown in Figure 1 was obtained from the Protein Data Bank (PDB) database. Structural data for minoxidil and finasteride compounds were accessed through the PubChem database. A total of 32 test ligands whose structures are displayed in Table 1 were obtained from various previous research articles [14-16].

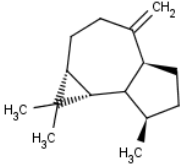
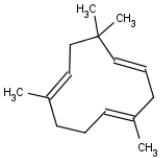
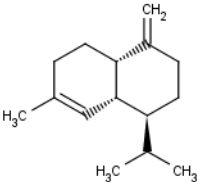
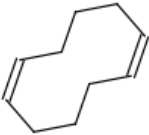
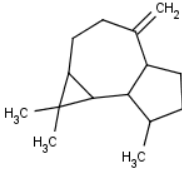
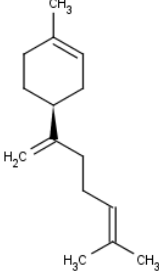
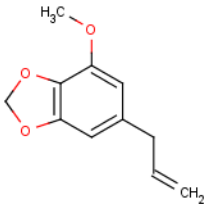
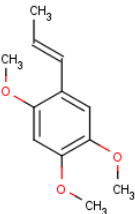
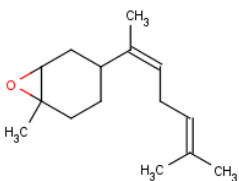
Ligand Structure Preparation

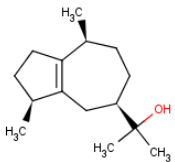
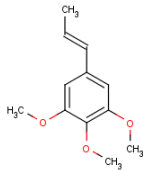
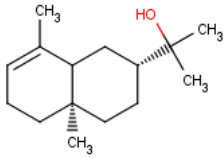
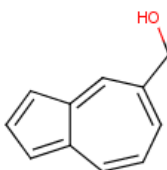
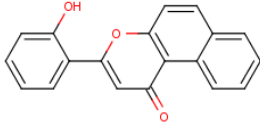
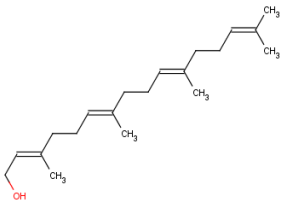
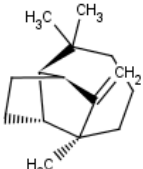
The tested ligand molecules consisted of 34 compounds comprising 2 native ligands (Minoxidil and Finasteride) and 32 ligands identified in nutmeg from several literature sources [16], [17] as shown in Table 1. The 3D ligand structures were obtained from <https://pubchem.ncbi.nlm.nih.gov/> and subsequently optimized using Avogadro software with geometry optimization using MMFF94 with 10,000 steps. The ligands were further prepared using AutoDockTools 4 software for water molecule removal, hydrogen atom addition, Gasteiger charge addition, non-polar hydrogen merging, and rotational bond configuration for flexibility. The ligand files were then saved in pdbqt format.

Table 1. Two Dimentional of Minoxidil, Finasteride, and Ligand Test

ID	Structure	Compound
1		Minoxidil
2		Finasteride
3		(+)-4-Carene
4		gamma-Terpinene
5		2-Cyclohexen-1-ol
6		Linalol
7		trans-4-methoxy thujane
8		Isoborneol
9		Terpinen-4-ol

10		L-alpha-Terpineol
11		cis-4-methoxy thujane
12		Geraniol
13		Safrole
14		alpha-Cubebene
15		Copaene
16		Methyleugenol
17		Caryophyllene
18		Bergamotene

19		Alloaromadendrene
20		Humulene
21		gamma-Muurolene
22		1,6-Cyclodecadiene
23		Aromadendrene
24		beta-Bisabolene
25		Myristicin
26		Asarone
27		trans-Z-alpha-Bisabolene epoxide

28		Guaiol
29		Isoelemicin
30		2-Naphthalenemethanol
31		5-Azulenemethanol
32		1-H-Naphtho[2,1-b] pyran-1-one, 3-(2- hydroxyphenyl)
33		Geranylgeraniol
34		Longifolene

Protein Structure Preparation

The target protein was the crystal structure of androgen receptor ligand-binding domain in complex with minoxidil (PDB: 4K7A) obtained from <https://www.rcsb.org/>. This receptor was selected as the target due to its role in hair follicle regulation through interaction with dihydrotestosterone (DHT). 4K7A activation by DHT causes follicular miniaturization, contributing to hair loss [18]. Protein structure preparation was performed using AutoDockTools software. Preparation steps included native ligand separation from target protein, water molecule removal, hydrogen atom addition, Gasteiger charge addition, and

non-polar hydrogen merging. The target protein was then saved in pdbqt format.

Molecular Docking Method Validation

Molecular docking method validation was performed by redocking minoxidil to the target protein separated from androgen receptor using AutoDock Tools 1.5.7. The validation process involved redocking native minoxidil ligand to androgen receptor 4K7A after separating native DHT and minoxidil ligands from protein receptor, then adjusting grid box position on native minoxidil ligand. Subsequently, redocking was performed to determine root-mean-square deviation (RMSD) values by

superimposing separated native ligand before docking with redocked native minoxidil ligand. This method was considered successful if RMSD values obtained were $\leq 3\text{\AA}$ [19].

Docking Simulation

Molecular docking was performed using AutoDock4.0 (AD4.0) and AutoDockTools (ADT). Grid parameters were set with center coordinates -2.562, 0.432, -6.758 and grid size $20 \times 20 \times 20$. Search parameters included 100 GA runs and population size 150. AutoDock4.2 parameters used Parameter_File AD4.1_bound.dat. Results were analyzed and visualized using Biovia Discovery Studio software.

Discovery Studio was used for visualization to determine hydrogen bond distances ($\text{\AA}$) and nearest amino acid residues from docking results.

Molecular Dynamics Simulation

Molecular dynamics simulation evaluated interactions between compounds and androgen receptor 4K7A, using finasteride and minoxidil as reference ligands. Ligand parameters and topology were prepared using ACPYPE [17], while Ewald particle mesh method was applied for electrostatic force calculations [21], [22]. The system was neutralized with Cl^- and Na^+ ions, and cubic TIP3P water model was used as solvent. The simulation protocol consisted of energy minimization, followed by NVT equilibration (100 ps, 310 K) and NPT equilibration (100 ps, 310 K, 1 bar) phases. The production run was performed under NPT ensemble conditions with 2 fs timestep for 25 ns using GROMACS 2024.2 [23], then run with 2 fs timestep for 25 ns using GROMACS 2024.2. Post-simulation analysis included Root Mean Square Deviation (RMSD) for protein-ligand complex stability evaluation, Root Mean Square Fluctuation (RMSF) for protein backbone flexibility identification, and Solvent Accessible Surface Area (SASA) for assessing protein surface area changes during ligand interaction.

Table 2. Results of Molecular Docking Method Validation

Protein	Compound	Binding Energy (kcal/mol)	RMSD	Hydrogen Bond Distance (Å)	Bound Amino Acids	Nearest Amino Acids
4K7A	Minoxidil Native	-4.85	2.25	2.94, 3.63	HIS789, GLU793	LYS861, LEU797,

Result and Discussion

The binding analysis results between minoxidil and androgen receptor are presented in Table 2. Native minoxidil formed hydrogen bonds with androgen receptor at amino acid residues HIS789 and GLU793 with hydrogen bond distances of 2.94 $\text{\AA}$ and 3.63 $\text{\AA}$, respectively. The resulting binding energy was -4.85 kcal/mol. The nearest identified amino acid residues were LYS861, LEU797, TYR857, and GLN858.

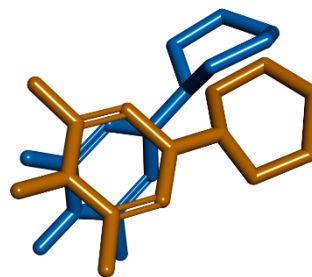


Figure 1. Overlay of docked pose of minoxidil with that of the co-crystallized ligand of 4K7A

Based on visual analysis results shown in Figure 1, minoxidil molecular docking validation demonstrated good agreement with the cocrystal ligand from 4K7A structure with RMSD value of 2.25 $\text{\AA}$. The RMSD value obtained was less than 3 $\text{\AA}$, indicating that the molecular docking method used was valid and reliable for predicting ligand docking poses [19]. Compared to similar research, there were differences from results reported by Abdurahman, who explained that minoxidil formed hydrogen bonds with amino acid residues SER865 and GLU793 [24], while as shown in Table 2, hydrogen bonding in this molecular docking occurred at residues HIS789 and GLU793. Another study by Albalawi et al. showed that minoxidil formed hydrogen bonds with GLU793, identifying nearest amino acid residues as LEU862, LYS861, and TYR857, with two residues identical to this research: LYS861 and TYR857 [18].

Based on available data, GLU793 residue was identified as forming hydrogen bonds with minoxidil in all three studies, indicating the significance of this residue in minoxidil-androgen receptor interaction. Variations in amino acid residues forming hydrogen bonds and other nearest residues influence binding energy and ligand-receptor complex stability. This aligns with Albalawi's research showing that differences in amino acid residues forming hydrogen bonds with androgen receptor are factors affecting binding energy [18].

Molecular interaction analysis of protein-ligand complexes in Table 3 demonstrates that hydrogen bonds play important roles in molecular interactions at AGA receptors, where hydrogen bonds are key in determining protein-ligand binding affinity and selectivity [25]. GLU793 residue dominance as the primary hydrogen bond former in 13 of 34 analyzed compounds shows the importance of specific electrostatic interactions. Optimal hydrogen bond distances observed in the 1.66-2.04 Å range are consistent with findings that hydrogen bond interaction patterns at protein-ligand interfaces require specific geometric requirements for optimal stability [26]. Binding site characteristics in this research were dominated by hydrophobic residues LEU797 (76.5%), LEU862 (73.5%), and LYS861 (82.4%).

This interaction pattern confirms that combinations of selective hydrogen bonds with polar residues and hydrophobic interactions with leucine clusters form the basis of effective molecular recognition in protein-ligand systems.

Structure-activity relationship (SAR) analysis showed terpenoid compounds with Geranylgeraniol (-7.00 kcal/mol) had good binding strength nearly matching Finasteride affinity (-7.19 kcal/mol), indicating terpenoid potential as anti-alopecia lead compounds. Geranylgeraniol was selected for molecular dynamics analysis as it demonstrated the highest binding affinity among nutmeg compounds and formed a hydrogen bond with GLU793 at 1.77 Å with favorable interactions at key binding site residues, while other nutmeg compounds showed considerably lower binding energies with the second-best being ID: 32 at -6.19 kcal/mol. Structural advantages of terpenoids, particularly those with hydroxyl groups, demonstrate optimal complementarity with binding sites rich in hydrophobic residues and hydrogen bond donors/acceptors, consistent with understanding that terpenoid cyclases catalyze the most complex chemical reactions in biology where more than half of substrate carbon atoms undergo bond and hybridization changes [27].

Table 3. Molecular Docking Result

ID Senyawa	Energi Ikatan (kcal/mol)	Jarak Ikatan Hidrogen (Å)	Asam Amino pada Ikatan Hidrogen	Residu Asam Amino Terdekat
2	-7.19	2.84, 3.62	SER865, GLU793	GLN858, LEU862, LEU797, LYS861, TYR857, ARG854, HIS789, LEU790
33	-7.00	1.77	GLU793	LEU862, TYR857, ARG854, LYS861, LEU797
32	-6.19	2.98	GLN858	GLU793, LEU862, LYS861, LEU797
18	-5.77	-	-	GLU793, SER865, LEU790, GLN858, LEU797, LYS861, LEU862, HIS789
15	-5.58	-	-	SER865, GLU793, LEU862, LEU797, GLN858, ASP864, LYS861
24	-5.44	-	-	TYR857, GLN858, LYS861, SER865, TRP796, LEU862, GLU793, LEU797

28	-5.43	1.66	GLU793	LEU797, LYS861, TYR857
30	-5.29	1.96	GLU793	LEU797, LYS861, TYR857
14	-5.11	-	-	GLU793, LEU797, LYS861, TYR857
21	-5.03	-	-	GLU793, LEU797, LYS861
23	-5.01	-	-	GLU793, LEU797, LYS861, TYR867
26	-4.9	2.76, 3.2	GLU793	LEU797, LEU862, TYR857, LYS861, LYS861, SER865
1	-4.85	2.94, 3.63	HIS789, GLU793	LYS861, LEU797, TYR857, GLN858
25	-4.85	-	-	LEU862, LEU797, GLU793, SER865, LYS861
6	-4.84	1.81	GLU793	LEU862, LEU797, SER865, LYS861
31	-4.83	1.92	GLU793	LEU862, SER865, LYS861
19	-4.82	-	-	LYS861, LEU862
29	-4.78	-	-	LEU862, LYS861, TYR857
17	-4.75	-	-	LYS861, TYR857, GLN858, LEU797, GLU793, LEU862, SER865
12	-4.71	1.97	GLN858	LEU862, LEU797, LYS861
20	-4.69	-	-	GLU793, SER865, LYS861, LEU 862, LEU797, GLN858, TYR857
27	-4.69	-	-	TYR857, SER865, GLU793, LEU862, LEU797, GLN858, LYS861
34	-4.68	-	-	GLN858, LEU862, LYS861, LEU797
16	-4.58	2.04	GLN858	LEU797, LYS861, TYR857, LEU862
7	-4.46	-	-	LEU862, LYS861
9	-4.42	1.97	GLN858	LEU862, LYS861
11	-4.36	-	-	LYS861, LEU797
10	-4.31	1.75	GLU793	LYS861, TYR857
13	-4.3	2.16	GLN858	LYS861, LEU797, LEU862
8	-4.23	1.88	GLU793	LYS861
22	-3.85	-	-	LYS861
3	-3.76	-	-	LYS861, LEU797
4	-3.73	-	-	GLU793, LYS861, LEU862
5	-3.55	1.88	GLU793	LEU862, LYS861

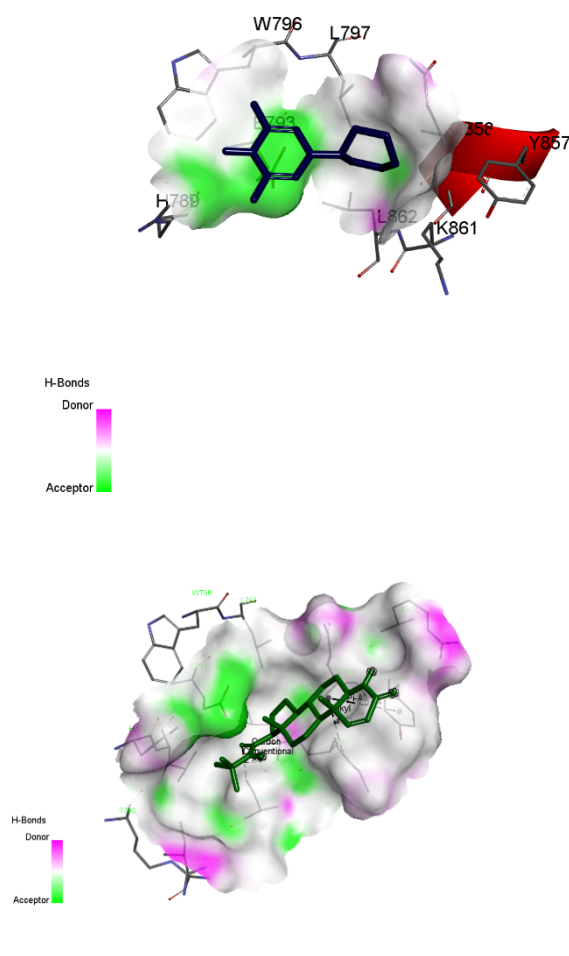
Minoxidil (ID: 1), the standard topical therapy for AGA, demonstrated weaker binding energy (-4.85 kcal/mol) with two hydrogen bonds at HIS789 (2.94 Å) and GLU793 (3.63 Å) residues **Figure 2** (B). Nearest amino acid residues included LYS861, LEU797, TYR857, and GLN858. This result is consistent with the fact that Minoxidil does not work through direct 5 α -reductase inhibition, but rather through KATP channel activation [28]. Research shows that human hair follicles contain two forms of KATP channels, with only one being sensitive to minoxidil [29]. Minoxidil activates mitochondrial KATP channels with

EC50 7.3 μ M, which is 25-fold more sensitive compared to sarcolemma channels [30]. This vasodilator mechanism enhances hair follicle perfusion and prolongs anagen phase, fundamentally different from enzymatic inhibition performed by Finasteride.

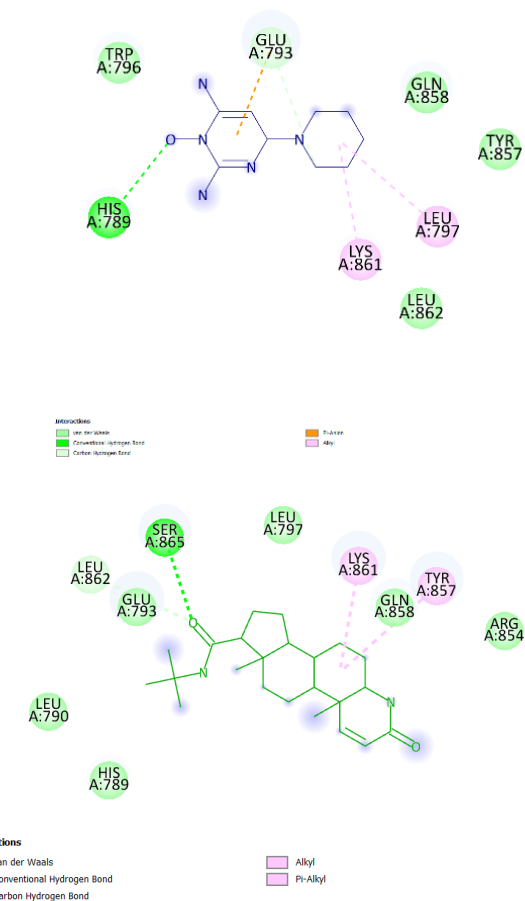
Further analysis of **Table 3** reveals that GLU793 is a crucial residue that interacts with all three test compounds. Crystallographic research by Xiao et al. (2020) identified this glutamate residue (E57 in their structural numbering) along with Y91 as important catalytic residues in 5 α -reductase enzymatic mechanism [31]. E57Q and Y91D mutations

proved to significantly reduce enzyme activity, showing the importance of these residues in catalysis [32]. Recent virtual screening studies identified that Trp53, Glu57, Tyr91, Arg94, Tyr98, Asn160, Asp164, Asn193, Phe194, Glu197, Phe223, and Leu224 residues are common residues that interact with 5 α -reductase inhibitors including finasteride [33]. This interaction pattern similarity between Finasteride and Geranylgeraniol shown in Figure 2 (A) and (C) indicates that Geranylgeraniol potentially occupies the same

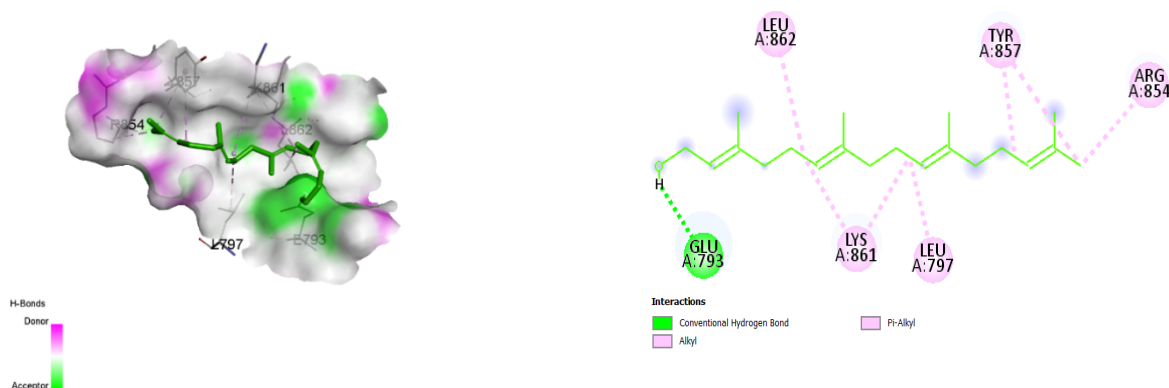
active site and potentially has similar inhibition mechanisms. Interestingly, although Finasteride forms two hydrogen bonds, Geranylgeraniol with one shorter hydrogen bond (1.77 Å vs 2.84 Å and 3.62 Å) can achieve nearly equivalent binding energy. This shows that individual hydrogen bond strength and hydrophobic interactions with residues like LEU862, LEU797, and electrostatic interactions with LYS861 and ARG854 contribute significantly to complex stability.



A



B



C

Figure 2. Molecular docking visualization between androgen receptor and (a) Minoxidil, (b) Finasteride, and (c) compound 33 (Geranylgeraniol)

Geranylgeraniol is a C20 isoprenoid found naturally in fruits, vegetables, and grains [34]. As a food substance, Geranylgeraniol is categorized as 'Generally Recognized as Safe' (GRAS) by the FDA [33]. In the mevalonate pathway, GGOH functions as a precursor for vitamin K2 MK-4 biosynthesis, coenzyme Q10, and various proteins undergoing prenylation [36] [37]. Several supplement formulations have begun using Geranylgeraniol with claims that it can act as a 5α -reductase inhibitor that maintains testosterone [38]. Compared to finasteride, which has risks of systemic side effects, Geranylgeraniol has several potential advantages including GRAS status indicating good safety profile, natural occurrence in food,

additional benefits for mitochondrial health and cellular energy production, and can be administered both topically and orally.

Based on RMSD data displayed in Figure 3, stability analysis of protein-ligand complexes during 25 ns molecular dynamics simulation showed characteristic stability patterns for all three test compounds. RMSD (Root Mean Square Deviation) values are commonly used measures for comparing different conformations of specific molecular systems [39] and specific conformational stability is typically indicated by structural changes or deviations over certain time periods [40].

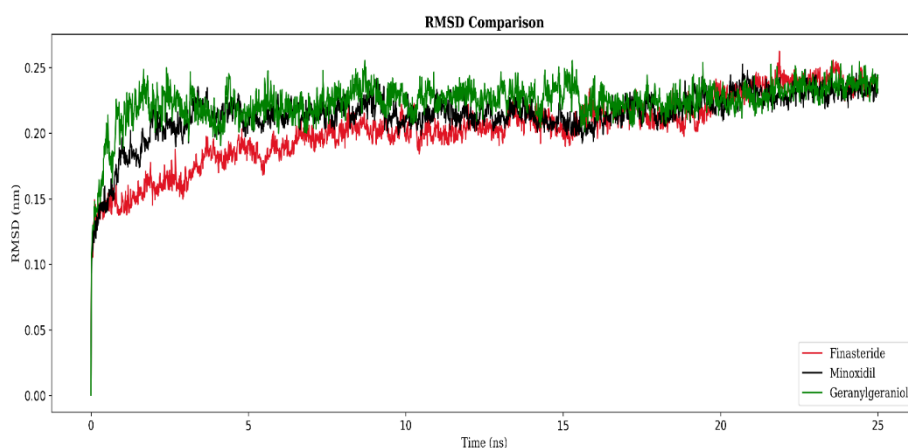


Figure 3. Root Mean Square Deviation (RMSD) Analysis of Androgen Receptor 4K7A Protein Structure in Complex with Ligands During 25 ns Simulation

Finasteride (red line) showed the most stable RMSD pattern with convergence values around 0.20-0.22 nm throughout the simulation period. This stability profile reflects finasteride's ability to maintain consistent conformation in complex with androgen receptor. This high conformational stability is consistent with finasteride characteristics as an effective competitive inhibitor, where stable and consistent interactions with enzyme active site are required for optimal biological activity. The relatively flat RMSD pattern after initial nanoseconds indicates that the finasteride-protein complex has reached stable conformational equilibrium.

Minoxidil (black line) showed different RMSD characteristics with values ranging between 0.21-0.23 nm and demonstrated greater fluctuations compared to finasteride. Minoxidil's RMSD profile indicates higher conformational dynamics in the protein-ligand complex. This can be explained by fundamental differences in minoxidil's mechanism of action, which does not work as a direct 5 α -reductase inhibitor but through KATP channel activation. Additionally, the relatively low binding energy observed in molecular docking results in Table 3 is also a factor influencing these high fluctuations [41]. Greater fluctuations in RMSD values indicate that minoxidil has the ability to adopt various conformations in the binding site,

which may relate to different mechanisms of action and lower affinity toward target protein.

Geranylgeraniol (green line) showed RMSD patterns with the highest values ranging between 0.23-0.25 nm throughout the simulation. Although geranylgeraniol's RMSD values were higher compared to the other two compounds, RMSD values from all three compounds ranging between 0.2-0.3 nm (2-3 Å) are still acceptable and considered to have stable binding [42], [43]. This flexibility may provide advantages for geranylgeraniol's biological activity, as it can facilitate interactions with various residues in the protein active site.

The different RMSD patterns of the three compounds reflect unique interaction characteristics of each with androgen receptor. Finasteride with the lowest and most stable RMSD shows strong and specific interactions, consistent with the most negative binding energy (-7.19 kcal/mol). Geranylgeraniol with slightly higher but still stable RMSD demonstrates the ability to form effective complexes with target protein, supported by binding energy nearly equivalent to finasteride (-7.00 kcal/mol). Minoxidil with RMSD patterns showing greater fluctuations reflects weaker interactions and different mechanisms of action, consistent with lower binding energy (-4.85 kcal/mol) (Table 3).

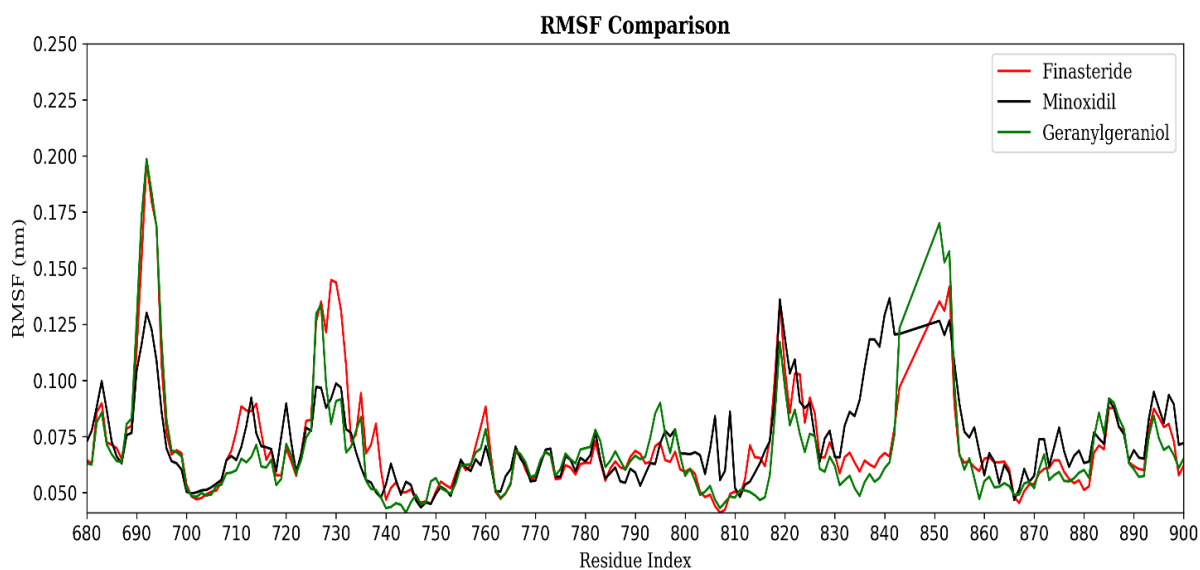


Figure 4. Root Mean Square Fluctuation (RMSF) Analysis of Androgen Receptor 4K7A Protein Backbone for Main Chain Flexibility Evaluation

The Root Mean Square Fluctuation (RMSF) analysis of the androgen receptor protein backbone during 25 ns molecular dynamics simulation presented in Figure 4 reveals distinct fluctuation patterns for the three test ligands. The RMSF graph demonstrates several regions with significant fluctuations, indicating structural dynamic changes in the protein during ligand interactions. At residue index 690, finasteride and geranylgeraniol exhibit nearly identical fluctuation patterns with the highest RMSF values of approximately 0.2 nm, while minoxidil shows lower fluctuations at 0.13 nm. Based on previous studies, this region encompasses residue 693, which has been identified as one of the loop regions in the androgen receptor protein structure. This loop possesses inherently high flexibility, explaining the substantial fluctuations observed [24].

A significant difference is observed in the region around residue index 730, where finasteride demonstrates the highest RMSF value (0.145 nm). This region is adjacent to residues 726 and 729, which have also been reported as parts of protein loops that naturally possess high flexibility and are responsive to

ligand binding. In the region around residue index 820, all three ligands show increased fluctuations with similar patterns. This can be attributed to this region's proximity to residue 796, which is also known to be part of a loop structure. This loop likely undergoes similar conformational changes due to interactions with all three ligands.

In the region around residue indices 840-850, geranylgeraniol induces the highest fluctuations up to 0.17 nm in the protein structure. The high fluctuations induced by geranylgeraniol indicate significant structural flexibility, which may be important for its biological activity. Geranylgeraniol from nutmeg demonstrates a distinct capability as a natural ligand to induce higher fluctuations in several regions, including those correlated with identified protein loops, compared to finasteride and minoxidil. Residues or groups of residues exhibiting higher RMSF levels indicate increased flexibility related to enhanced potential for ligand molecule interactions, while lower RMSF fluctuations signify reduced flexibility, thereby diminishing interaction potential [44].

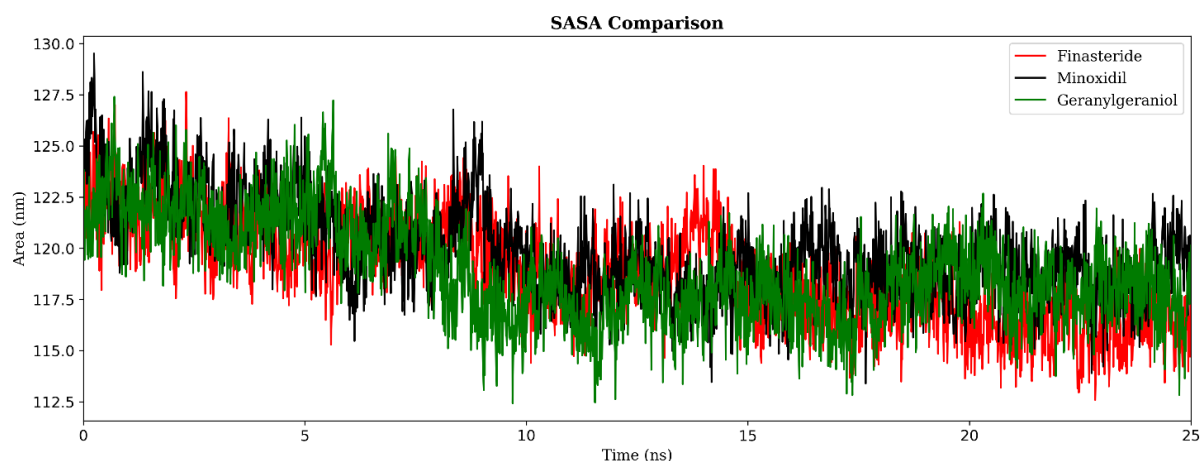


Figure 5. Solvent Accessible Surface Area (SASA) Analysis of Androgen Receptor Protein 4K7A

Solvent Accessible Surface Area (SASA) analysis is employed to measure the surface area of the androgen receptor protein exposed to solvent [45] and to evaluate the stability of protein-ligand complexes during 25 ns molecular dynamics simulation. The SASA graph in Figure 5 demonstrates dynamic changes in the protein surface with the three ligands, where finasteride, minoxidil, and

geranylgeraniol exhibit fluctuations within the range of 112.5-130 nm² throughout the simulation. During the initial phase (0-5 ns), all three complexes show relatively high SASA values with large fluctuations, indicating systems still undergoing conformational adjustments. A decreasing trend in SASA values is observed from the middle to the final phases of simulation, with finasteride showing

the most significant decrease, followed by geranylgeraniol which demonstrates more stable SASA reduction compared to minoxidil, particularly during the 10-15 ns phase where geranylgeraniol reaches the lowest value of approximately 112.5 nm². Lower SASA values indicate that the complex system becomes more stable and compact. This demonstrates that geranylgeraniol from nutmeg possesses better complex stability compared to minoxidil, although it remains below finasteride. Based on these results, it can be concluded that the compound from nutmeg has potential as an androgen receptor inhibitor with reasonably good complex stability.

Conclusion

This computational study successfully identified geranylgeraniol from nutmeg (*Myristica fragrans* Houtt.) as a promising natural compound for androgenetic alopecia treatment through androgen receptor inhibition. Molecular docking analysis revealed that geranylgeraniol exhibits strong binding affinity (-7.00 kcal/mol) to the androgen receptor, approaching the efficacy of finasteride (-7.19 kcal/mol) and substantially outperforming minoxidil (-4.85 kcal/mol). The compound demonstrates favorable binding characteristics through hydrogen bond formation with GLU793 and favorable interactions with key residues in the receptor binding site.

Molecular dynamics simulation over 25 ns confirmed the stability of the geranylgeraniol-androgen receptor complex, with consistent RMSD values (0.23-0.25 nm) indicating maintained binding throughout the simulation period. The RMSF analysis revealed that geranylgeraniol induces strategic structural flexibility in specific protein regions, particularly in loop structures, which may enhance its biological activity. Furthermore, SASA analysis demonstrated improved complex compactness and stability compared to minoxidil, suggesting better therapeutic potential. However, the 25 ns simulation duration may not be sufficient to evaluate true complex stability and capture rare conformational events. Future work should consider extending the simulation time to

provide more comprehensive evaluation of binding stability and long-term protein-ligand interactions.

These computational findings provide strong evidence for geranylgeraniol's potential as an effective natural alternative for androgenetic alopecia treatment. The compound's comparable efficacy to finasteride, combined with its natural origin, makes it an attractive candidate for further experimental validation. However, in vitro and in vivo studies are essential to confirm these computational predictions and establish the compound's safety profile and therapeutic efficacy in clinical applications.

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