

ANTIALOPECIA ACTIVITY EXPLORATION BY *In-silico* ANALYSIS OF BIOACTIVE COMPOUNDS FROM *Sansevieria* *trifasciata* PRAIN LEAVES THROUGH 5 α -REDUCTASE INHIBITION

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ABSTRACT

Numerous reports link hair loss to alopecia, a major concern in baldness treatment. Identifying new, effective 5 α -reductase inhibitors is crucial for better alopecia treatment. This study explores the *Sansevieria trifasciata* Prain fraction's potential as an inhibitor based on docking and molecular dynamics. Oliveramine (-9.14 kcal/mol) and Methyl pyropheophorbide A (-7.93 kcal/mol) exhibit the best affinity, interacting with critical residues Arg114 and Phe223. MM-PBSA analysis confirms their superiority over finasteride, with binding affinities of -146.472 kJ/mol and -120.004 kJ/mol, respectively. Methyl pyropheophorbide A shows stability, cohesiveness, and intense interaction similar to NADP-DHF, supporting further *in vitro* studies for alopecia treatment evaluation.

Keywords: Alopecia, Docking, Dynamics Studies, 5 α -reductase-2, *Sansevieria trifasciata* Prain.

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INTRODUCTION

Plant-derived pharmaceuticals are being developed due to the public's interest in herbal treatment reuse. *Sansevieria trifasciata* Prain shows potential as a medication containing chalcone chemicals, steroidal saponins^{1,2}, isoflavonoids³, glycosides⁴, and alkaloids.⁵ *In vivo*, studies confirm its ability to stimulate hair growth.⁶⁻⁸ In our previous studies, rabbits with alopecia were subjected to *in vivo* assays, and obtained extracts and fractions could significantly stimulate new hair growth and improve the anagen/telogen ratio.⁹ Further exploration of *S. trifasciata*'s molecular mechanisms is needed to investigate its anti-alopecia activity. High androgen expression,¹⁰ including testosterone and 5 α -DHT, is associated with androgenic alopecia, characterized by patterned hair loss due to shortened anagen phase.¹¹ Three main targets in alopecia treatment are 5 α -reductase, androgen receptors, and growth factors.¹² Previous research identified *S. trifasciata*'s potential in preventing alopecia by interacting with androgen receptors,^{13,14} but the molecular mechanism remains unclear. Thus, we investigated the molecular interactions between *S. trifasciata* and another target, 5 α -reductase. The 5 α -reductase enzyme transforms testosterone into dihydrotestosterone (DHT). Linoleic acid, palmitic acid,¹¹ and stigmasterol¹⁵ are known to inhibit this enzyme. *S. trifasciata* contains these compounds, suggesting potential interaction with 5 α -reductase to prevent baldness.¹⁶ Hence, in this study, we focused on evaluating the ability of *S. trifasciata* to inhibit 5 α -reductase through *in-silico* investigation combining molecular docking and dynamic simulation.

EXPERIMENTAL

Instrumentation

The docking and dynamic simulation were performed using a computer with hardware featuring an Intel® Core i5-8500 processor @ 4.30GHz (6 CPUs), 4 GB RAM, 120 GB SSD, 2TB HDD, and GTX 1080 Ti VGA Intel Graphics.

Protein dan Ligand Structure Preparation

We select the crystallographic structure of 5-reductase 2 (5 α R-2) with a Protein Data Bank ID of 7BW1.¹⁷ This study employed this structure based on bound inhibitor (finasteride as a native ligand) and high-

resolution x-ray. Water molecules and associated ligands got separated from the system. By employing AutoDock Tools v.1.5.6, the target protein was protonated by incorporating polar hydrogen atoms and Kollman charges.¹⁸ Additionally, the structures of identified compounds (ligands) were gathered from the PubChem database.¹⁹ Finally, hydrogen atoms were incorporated into all ligand structures, and the Gasteiger charges were modified using AutoDock Tools.²⁰

Molecular Docking Study

To investigate the binding affinity and interactions of the identified compounds in *S. trifasciata*, the Lamarckian genetic technique with a population size of 100 was applied in the AutoDock program.¹⁸ To verify the docking parameters, finasteride was redocked to the 5 α R-2. The validated procedures resulted in a root mean square deviation (RMSD) below 2 Å²¹ were then used for docking all identified ligands in the *S. trifasciata* fraction. The binding site was calculated using the cubic shape grid area following the finasteride position. The grid area was set to 60 × 60 × 60 Å with 0.375 Å spacing. The Discovery Studio Visualizer explored and presented the protein's hydrophobic interactions and hydrogen bonds with its ligands.

Molecular Dynamics Simulation

The 5 α R-2-ligand systems were simulated using GROMACS 2021.3 software.²² AMBER99SB-ILDN force field was applied for protein topology,²³ while the General Amber force field was used for ligand topology²⁴ via Antechamber in AmberTool 2021 with ACPYPE.²⁵ The system was solvated using the TIP3P water model, maintaining a minimum distance of 10 Å around the complex, and neutralized with Na and Cl ions. The minimization step consisted of three phases involving conjugate gradients and steepest descents. Gradual heating to 310K was performed in an NVT ensemble with a time step of 0.0005 ps at intervals of 50 ps.²⁶ The particle mesh Ewald approach was used to simulate electrostatic interactions and adjust the 1.2 nm cut-off for van der Waals energy terms.²⁷ Complex stability was assessed through RMSD, RMSF, Rg, SASA, and PCA analysis. Finally, the complex systems' binding energies were determined using the MM/PBSA theory in the g_mmpbsa packages.²⁸

RESULTS AND DISCUSSION

Molecular Docking

Molecular docking analyses predicted the preferred binding modes of a ligand to a protein based on binding affinity predictions and amino acid interactions. Lower binding energy indicated higher efficiency and more potent inhibition. Critical amino acid residues in 5 α R-2 were emphasized for their essential role in ligand interactions, establishing hydrogen bonds for strong stabilization. The best conformation of finasteride had an RMSD value of 1.59 Å (Fig.-1). The lower the RMSD value, the better the docking parameters' ability to mimic the finasteride X-ray crystallography conformation.

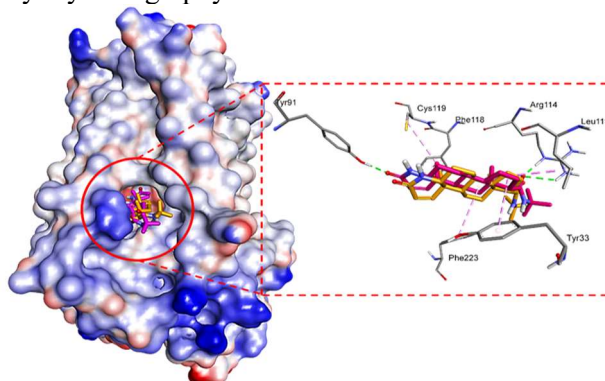


Fig.-1: Superimpose and Interaction of Docked Pose (Orange) and Cocrystallized (Pink) Finasteride Against 5 α R-2

From this work, finasteride has shown the strongest affinity to the 5 α R-2 enzyme compared to all the identified compounds in the *S. trifasciata* fraction. Finasteride and the NADPH cofactor complexed with Dihydrofinasteride (NADP-DHF) have binding affinities of -10.81 kcal/mol and -25.23 kcal/mol, respectively. Meanwhile, the identified compounds including Oliveramine, Methyl pyropheophorbide A,

(2S)-3',4'-Methylenedioxy-5,7-Dimethoxyflavane, Trichosanic acid, 1-Acetyl- β -carboline, Digiprolactone, and Methyl gallate exhibited lower binding affinity with energies of -9.14 kcal/mol, -7.93 kcal/mol, -7.18 kcal/mol, -7.14 kcal/mol, -6.01 kcal/mol, -5.90 kcal/mol, and -4.69 kcal/mol, respectively. The NADP-DHF interacted with 20 amino acid residues (Fig.-2) in the active sites of the 5 α R-2 with 14 hydrogen bonds, namely Lys35, Glu57, Tyr98, Asn102, Arg105, Arg114, Asn160, Asp164, Tyr178, Arg179, Asn193, Glu197, Tyr235, and Tyr333. It exhibits the closest amino acid residue interaction to finasteride, with two hydrogen bonds (Tyr91 and Arg114) identified in its interaction with 5 α R-2.

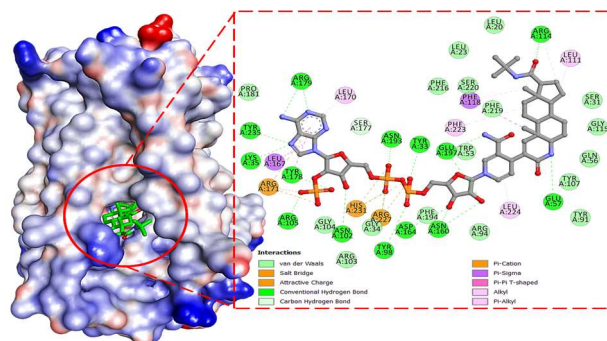


Fig.-2: Molecular Interaction of NADP-DHF with 5 α R-2

We are interested in analyzing the binding mode of Oliveramine (Fig.-3A) and Methyl pyropheophorbide A (Fig.-3B) because they have a binding energy close to finasteride. These compounds form hydrogen bonds with residues Arg114, and each compound includes new hydrogen bonds with residues Ser31, Trp201 and Ser220. Interestingly, the compounds from the *S. trifasciata* fraction revealed their ability to interact with three essential residues that contribute to forming hydrogen bonds at the 5 α R-2 binding sites, namely residues Glu57, Try91, and Arg114. Meanwhile, the hydrophobic interaction of finasteride was studied on crystallography and was found in residues Leu20, Leu23, Tyr33, Leu111, Phe118, and Phe223.¹⁷ Moreover, a previous study revealed that changing one aromatic residue Phe118 in the DHF-binding pocket to a leucine could significantly reduce 5 α R-2 activity by disrupting testosterone binding, implying that this residue plays an essential role in steroid substrate binding.²⁹ This study reveals the ability of oliveramin and Methyl Pyropheophorbide A to interact with serine and arginine residues. In the 5R-2 enzyme, serine acts as a catalyst, converting testosterone to DHT, while arginine residues at the active site aid in substrate binding and participate in the chemical conversion of testosterone to DHT. Binding to these residues inhibits 5R-2 activity by disrupting essential chemical bonding for catalytic processes.

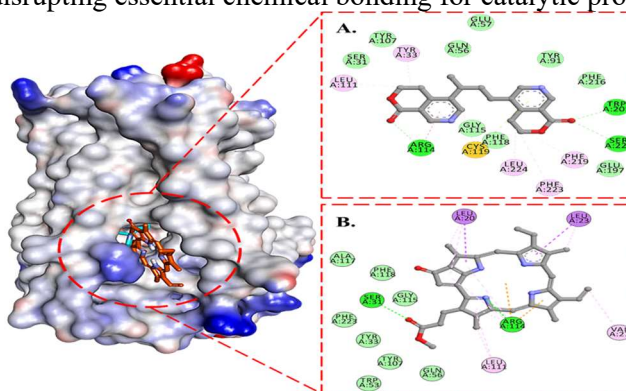


Fig.-3: Molecular Interaction of (A) Oliveramine and (B) Methyl Pyropheophorbide A with 5 α R-2

Molecular Dynamics Simulation

Root Mean-square Deviation (RMSD) and Root Mean-Square Fluctuation (RMSF)

The RMSD of the 5 α R-2 backbone and best compound in *S. trifasciata* subfraction (Methyl Pyropheophorbide A and Oliveramine) can be seen in Fig.-4A was compared to the finasteride and NADP-dihydrofinasteride (NADP-DHF). RMSD in system simulation compares folded and unfolded protein structures, indicating dynamic changes and assessing stability.³⁰ According to the simulation results,

finasteride and oliveramine have poor stability compared to the other compound. The Backbone of 5 α R-2 fluctuated more in the finasteride and Oliveramine complexes, with RMSDs of 0.549 nm and 0.464 nm, respectively. NADP-DHF and Methyl pyrophaeophorbide A exhibited similar stability after 40 ns of simulation. These two compounds remained stable throughout the simulation, with an average RMSD of 0.278 nm for NADP-DHF and 0.297 nm for Methyl pyrophaeophorbide A. These findings suggest that Methyl pyrophaeophorbide A can stabilize the 5 α R-2 system, as clearly illustrated by RMSD fluctuations that tend to be constant during the simulation.

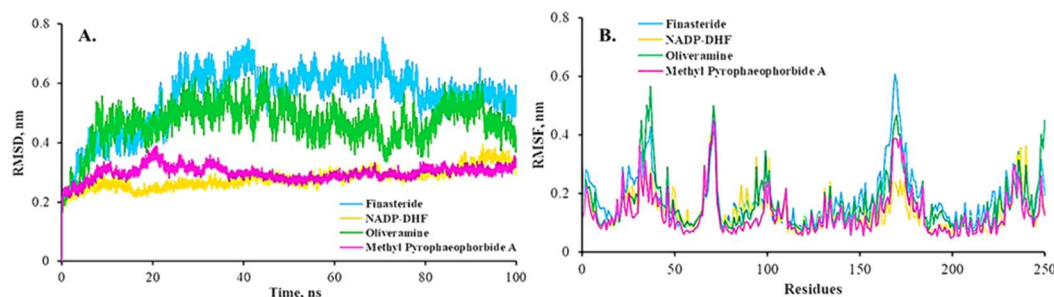


Fig.-4: Evaluation of (A) RMSD and (B) RMSF During Dynamic Simulations. The Complex of all Enzyme-Compounds has been Represented in Different Colors

The RMSF in the 5 α R-2 developed a variety of intensity values (Fig.-4B). RMSF displays residue coordinate fluctuations around the reference point during dynamic simulation, characterizing protein structure oscillation.³¹ No significant fluctuations in amino acid were observed in the RMSF profiles of each complex with 5 α R-2. High intensity oscillations were observed at residues Thr37, Gln71, and Gln169 around ~0.5-0.6 nm. Oliveramin and FNT compounds caused slightly different fluctuations than other compounds, especially in Thr37 and Gln169 residues. Uniquely, NADP-DHF tends to decrease the fluctuation of the Leu167-Glu175 residue during the simulation. Meanwhile, the RMSF values of other residues in 5 α R-2 display a similar pattern. These studies revealed that Methyl Pyrophaeophorbide A and Oliveramine did not change amino acid residue fluctuation.

Solvent Accessible Surface Area (SASA) and Radius Gyration (Rg)

During the simulation, variations in the solvent-accessible area serve as a crucial parameter to characterize the protein complex's folding and stability.³² Finasteride and oliveramine demonstrated an increase in the solvent-accessible area during the final 50 ns of the simulation (Fig.-5A). Meanwhile, NADP-DHF and Methyl Pyrophaeophorbide A areas tend to be constant during the simulation. Overall, the average area accessible to solvents were 144.07 nm², 143.45 nm², 140.67 nm², and 130.01 nm², respectively, for Oliveramine, Finasteride, NADP-DHF and Methyl Pyrophaeophorbide A.

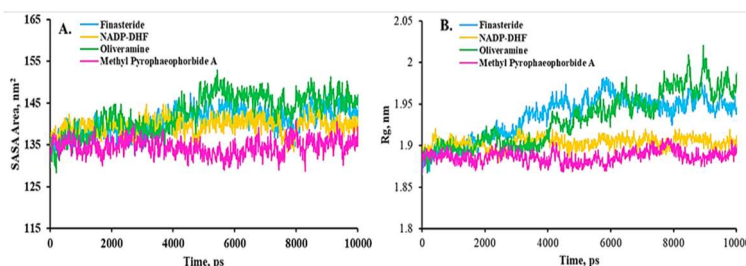


Fig.-5. Evaluation of (A) SASA and (B) Rg During Dynamic Simulations. The Complex of all Enzyme-Compounds has been Represented in Different Colors

During the simulation, the radius of gyration (Rg) of the protein was measured. Rg represents the scattering of atoms around the protein's axial direction. Ligand binding affects protein folding and stability, which can be observed through their pattern and Rg value. Stable protein folding is indicated by low and consistent Rg values during the simulation.³³ According to the graph (Fig.-5B), 5 α R-2 has a high Rg protein when interacting with Oliveramin and Finasteride, with an average of 1.963 nm and 1.958 nm, respectively. Both of these compounds showed an increase in Rg from 50 ns to the end of the simulation. Interestingly, Methyl

Pyropheophorbide A and NADP-DHF showed stable Rg protein during simulation with an average of 1,905 nm dan 1,871 nm. These findings indicate that the Methyl Pyropheophorbide A complex has the lowest Rg value while having higher compactness than other hit compounds.

Principal Component Analysis (PCA)

The protein's overall mobility trend is then assessed by employing PCA. PCA analyses trajectory data from atomic coordinate covariance matrices that describe degrees of freedom (DOF) of the protein recorded in the two eigenvectors. The eigenvalues represent the motion components, with larger eigenvalues indicating the larger-scale movement of protein residues. The cluster occupies less space during the simulation, revealing stable complexes.^{19,34} Surprisingly, the Methyl Pyropheophorbide A complex has a cluster configuration identical to NADP-DHF (Fig.-6). Meanwhile, the oliveramin and finasteride complexes show cluster areas opposite to the other complexes with larger sizes. Based on this analysis, it is clear that Pyropheophorbide A has the best stability when interacting with 5 α R-2.

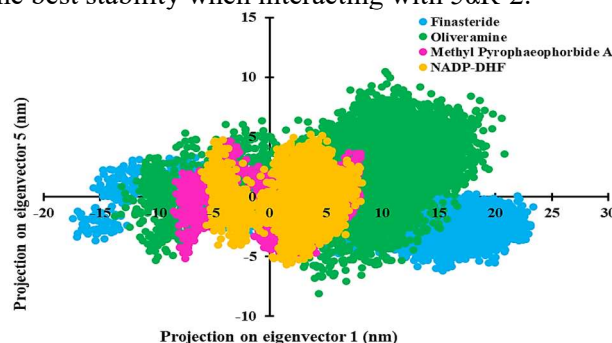


Fig.-6: Evaluation of 2D Projection of Trajectory During Dynamic Simulations. The Complex of all Enzyme-Compounds has been Represented in Different Colors

Binding Free Energy Enzyme-Compounds

The binding energy of all hit compounds was calculated from molecular dynamics simulation based on the MM-PBSA method shown in Table-1. MM/PBSA was applied to analyze the separate energies that influence the specific energy of system binding, uncovering significant interactions in the procedure, and aiding in drug development.³⁵ The NADP-DHF systems exhibited a total binding energy (ΔE_{Bind}) of -851.316 kJ/mol, which was lower compared to the system of all other compounds. DHF was strongly bound to 5 α -reductase 2 when it formed a complex with NADP. Methyl Pyropheophorbide A found the most negative binding energy with -146.472 kJ/mol, followed by Oliveramine at -120.004 kJ/mol, then finasteride (-115.820 kJ/mol).

Table-1: The Energies of all Complex Enzyme-Compounds during 100 ns Dynamic Simulation

Compounds	E_{VDW}	E_{Ele}	E_{PB}	E_{SASA}	ΔE_{Bind}
Methyl Pyropheophorbide A	-217.74 ± 27.36	-93.68 ± 25.88	189.27 ± 43.27	-24.32 ± 2.01	-146.47 ± 22.93
Oliveramine	-195.82 ± 15.69	-55.95 ± 18.97	149.58 ± 18.45	-17.81 ± 0.96	-120.00 ± 16.80
Finasteride	-187.45 ± 14.98	-12.37 ± 17.22	103.20 ± 23.98	-19.18 ± 0.94	-115.82 ± 14.45
NADP-DHF	-425.24 ± 32.03	-1328.18 ± 88.94	947.16 ± 89.93	-45.06 ± 1.44	-851.31 ± 44.66

The van der Waals (E_{VDW}), electrostatic (E_{Ele}), and SASA (E_{SASA}) energies were identified as crucial factors influencing favourable binding in all systems. Unfortunately, the hit compound exhibited less negative electrostatic energy compared to NADP-DHF. Additionally, polar solvation energy (E_{PB}) had an unfavourable contribution to the binding energy. These findings align with the stability analysis of all compounds, indicating that Methyl Pyropheophorbide A in *S. trifasciata* fraction has the potential to inhibit the activity of the 5 α R-2 enzyme.

CONCLUSION

Using computational techniques, we identified Methyl pyropheophorbide A as a potential metabolite from *S. trifasciata* to inhibit 5 α R-2 enzyme. Methyl pyropheophorbide A demonstrated strong binding affinity to 5 α R-2, comparable to finasteride and NADP-DHF, known inhibitors of this enzyme. This was evident

from its high binding energy and interactions with key amino acid residues in both rigid and flexible complexes. Furthermore, during dynamic simulation, this compound produced a stable complex, providing new opportunities for future research in finding novel drug candidates to treat alopecia by inhibiting the 5 α R-2 enzyme.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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