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Decoding Pumpkin Seeds for Hair Loss: A Computational Perspective

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Abstract:- Androgenetic alopecia (AGA) is a condition that affects both men and women who are genetically predisposed, resulting in a recognizable pattern of hair follicle shrinkage without scarring. It's estimated that around 80% of men and 50% of women will experience AGA at some point in their lives. The root cause of this condition can be linked to the action of the enzyme 5-alpha-reductase, responsible for converting testosterone into dihydrotestosterone (DHT), a more potent androgen. The accumulation of DHT in hair follicles ultimately leads to hair loss. The primary approach to treating AGA involves targeting the inhibition of 5-alpha-reductase. A recent trend in natural remedies involves the use of pumpkin seed for AGA treatment. In this study, the focus is on conducting virtual simulations and ADME/T (Absorption, Distribution, Metabolism, Excretion/Toxicity) analysis of active compounds found in pumpkin seed. The objective is to assess their interactions with the 5-alpha-reductase enzyme and visualize these interactions. This research aims to provide insights into the potential effectiveness of pumpkin seed as a therapeutic option for AGA, through computational evaluations of protein-ligand interactions.

Keywords:- *Androgenetic Alopecia, DHT, Finasteride, Pumpkin Seed, 5-Alpha Reductase.*

I. INTRODUCTION

Androgenetic alopecia (AGA) is a prevalent condition affecting both men and women, transcending mere aesthetic concerns to deeply impact one's self-perception and body image. This psychological phenomenon encompasses a spectrum of emotions, thoughts, behaviors, and perceptions tied to one's physical appearance. Despite its widespread occurrence, hair loss frequently gives rise to emotional distress, underscoring its significant influence. The process of AGA is characterized by elevated levels of dihydrotestosterone and the enzyme 5-alpha-reductase [2], with the latter converting testosterone into DHT in specific peripheral tissues [3]. DHT then binds to susceptible androgen receptors in hair follicles, triggering genetic pathways associated with follicular shrinking [4]. Standard treatments for AGA encompass minoxidil and finasteride. Minoxidil, available topically or orally, acts as a vasodilator, while orally administered finasteride inhibits 5-alpha-reductase, thereby reducing DHT levels [5]. However, the usage of finasteride is frequently marred by its adverse effects, including diminished libido and the onset of post-finasteride syndrome, leading to hesitancy among patients [6]. Psychological well-being, influenced by psychiatric conditions and personality traits such as neuroticism, plays a pivotal role in emotional equilibrium [7]. Given the formidable drawbacks associated with finasteride and patients' reluctance, the pursuit of alternative DHT-blocking strategies, like pumpkin seeds with established DHT-blocking attributes [8], becomes imperative. The primary constituents of pumpkin seeds, including beta-cryptoxanthine, beta-sitosterol, and linoleic acid, are currently under investigation. The core objective of this study revolves around evaluating and anticipating the intricate interactions between the active components of pumpkin seeds and the target protein, 5-alpha-reductase. Through this research endeavor, the aim is to uncover potential novel avenues for AGA treatment that can circumvent the undesirable effects linked to existing approaches.

II. MATERIAL AND METHODS

The in-silico investigation was conducted using a Hewlett Packard laptop equipped with hardware comprising an 8 GB RAM and an Intel i3 11th generation processor. The molecular docking analysis was executed using the AutoDock Vina tool[9], integrated within the PyRx software platform[10]. For protein preparation and the visualization of ligand-protein docking, PyMOL 2.4.1[11] was employed. To assess protein-ligand interactions, Ligplot+[12] was utilized. Additionally, the SwissADME webserver [13] was employed for conducting ADME/T studies.

- *Retrieval and Preparation of Ligand*

The active chemical constituents found in pumpkin seeds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in the format of Structural Data (SDF). The specific compounds selected for the in-silico analysis are detailed in Table 1. Ligand preparation was carried out through the utilization of the PyRx software. To optimize the energy of the ligands, the Open Babel tool [14] within the PyRx software was employed, using the MMFF94 force field for energy minimization. Subsequently, all ligands were converted into the AutoDock pdbqt format.

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Table 1 Chemical Constituents Found in Pumpkin Seeds Along with their Compound Identifiers and Chemical Characteristics are as Follows

S. NO.	Ligand name	Compound ID	Molecular weight (g/mol)
1	Beta-cryptoxanthine	5281235	552
2	Beta-sitosterol	222284	414
3	Linoleic acid	5280450	280

➢ *Preparation of the Protein of Interest*

The high-resolution 3D structure of 5-alpha-reductase was obtained from the Protein Data Bank (PDB) with the identifier 7BW1, accessible through the database link (https://www.rcsb.org/). To ready the protein for subsequent steps, water molecules were excluded to prevent interference during protein-ligand docking. PyMOL software was employed to introduce polar hydrogen atoms. Additionally, the attached finasteride molecule was detached to reset the binding site. Lastly, the protein's format was changed from PDB to pdbqt to enable its usage with AutoDock Vina.

➢ *Screening of the Ligands*

The virtual screening of the collected active compounds against the target protein was carried out using the AutoDock Vina tool integrated into PyRx software. Blind docking was conducted with a grid box size of (43.50 Å × 39.01 Å × 60.70 Å) and centered at coordinates (-33.23, 13.08, 29.71). The default setting of 8 exhaustiveness was applied. Following the screening analysis, active compounds with favorable docking scores were observed using PyMOL visualization software.

➢ *Analysis of Absorption, Distribution, Metabolism, and Excretion/Toxicity (ADME/T)*

The ADME/T analysis furnishes insights into the characteristics of absorption, distribution, metabolism, excretion, and toxicity exhibited by the examined ligands or chemical compounds. This analysis aims to eliminate undesired molecules lacking substantial drug-like qualities, streamlining the study's viability and efficiency. The canonical SMILES representation of the obtained compounds or hits was utilized to assess the pharmacological and physicochemical attributes via the SwissADME webserver (http://www.swissadme.ch/) [15]. Key parameters considered for pharmacological evaluation included molecular weight, count of hydrogen bond acceptors, count of hydrogen bond donors, topological polar surface area (TPSA) value, and LogS value.

➢ *Prediction of Biological Activity*

To forecast the biological effects of the selected molecules, the PASS web server (http://www.pharmaexpert.ru/passonline) [16] was utilized. Leveraging intricate atom neighbor descriptors, the PASS analysis assists in understanding a drug's impacts based solely on its molecular formula, implying that its biological function is entirely influenced by its chemical configuration.

III. RESULT AND DISCUSSION

➢ *Virtual Screening Result Analysis*

The 3D crystal structure of 5-alpha-reductase (PDB ID: 7BW1) was acquired from the protein data bank to conduct docking analysis. The primary active compounds found in pumpkin seeds were obtained from PubChem in Structural Data Format (SDF). The ligands, or active compounds, underwent screening against the intended target, 5-alpha-reductase, using the AutoDock Vina tool within PyRx. The docking scores of the compounds are detailed in Table 2.

Table 2 Docking Score of the Compounds Obtained after Virtual Screening

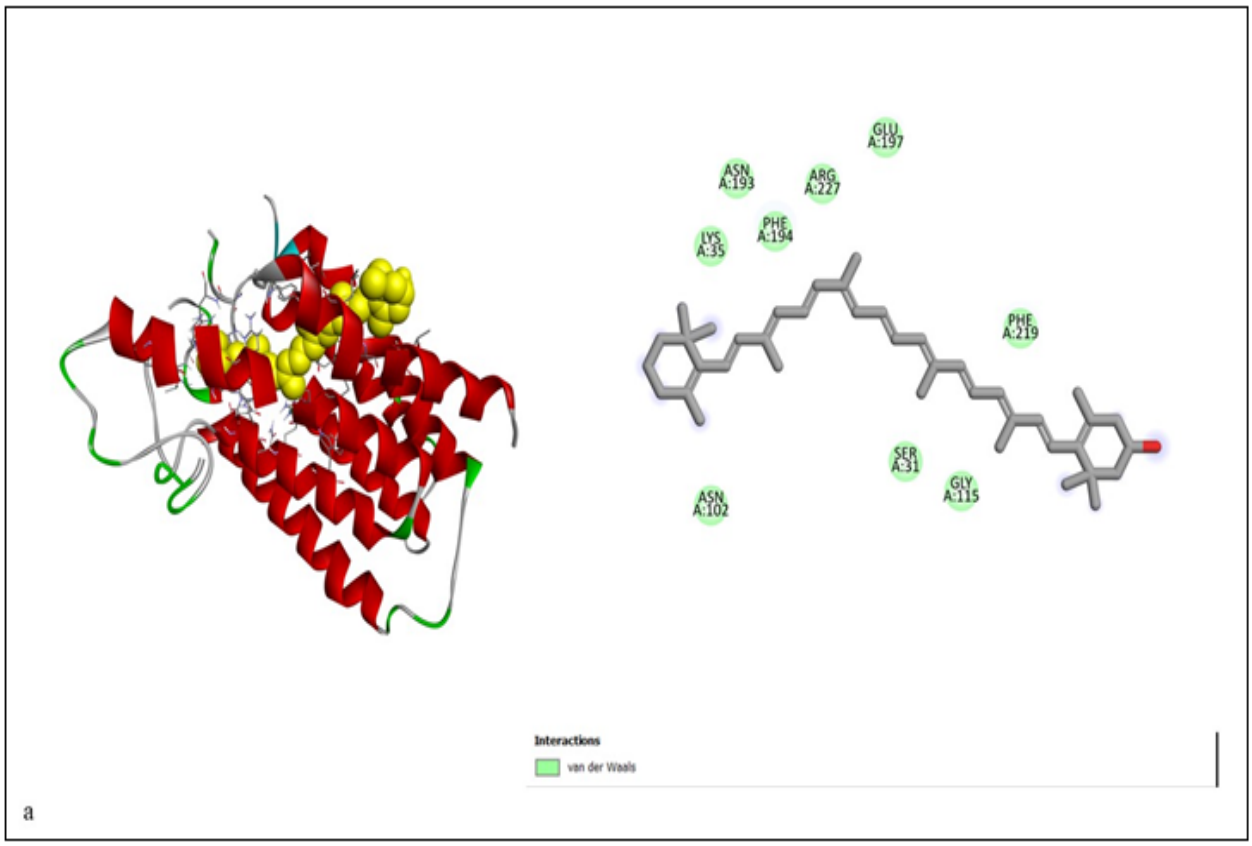
S. NO.	Ligand name	Docking score
1	Beta-cryptoxanthine	-10.5
2	Beta-sitosterol	-11.4
3	Linoleic acid	-6.5

➢ *Protein-Ligand Interaction Analysis*

Analysis of protein-ligand interactions was carried out using DiscoveryStudio software [17], focusing on identifying hydrogen bonds and Van der Waals interactions occurring between the protein's amino acids and the ligand. This analysis serves to forecast the binding affinity between the ligand and the target protein. Table 3 provides an overview of the diverse interactions demonstrated by the chosen compounds. The visual depiction of these interactions, both in 2D and 3D formats, can be found in Figure 2.

Table 3 Interactions Involving Hydrophobic Properties and Hydrogen Bonding between the Compound and the Active Site of the Target Protein

S. NO.	Ligand name	Van der Waals interaction	Hydrogen bond interaction
1	Beta-cryptoxanthine	Ser31, Lys35, Asn102, Gly115, Asn193, Phe194, Glu197, Phe219, Arg227	
2	Beta-sitosterol	Ser31, Tyr33, Gln56, Glu57, Arg94, Tyr107, Arg114, Gly115, Glu197, Phe216, Phe219, Leu224, Arg227	
3	Linoleic acid	Ser31, Tyr33, Glu57, Tyr107, Arg114, Gly115, Phe216, Ser220	Gln56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ASN A:193
ARG A:227
PHE A:194
LYS A:35
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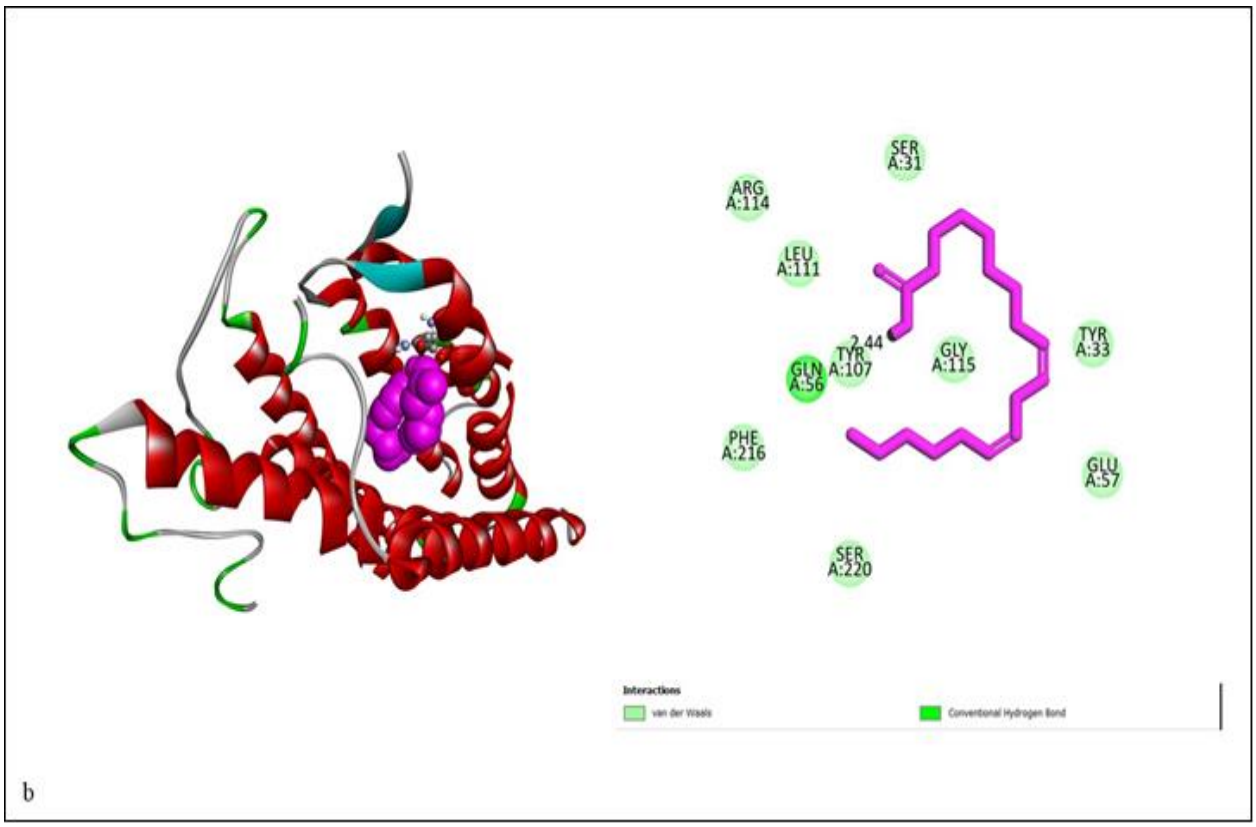
SER A:31
GLY A:115
ASN A:102
Interactions
van der Waals

a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ARG A:114
LEU A:111
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TYR A:107
GLY A:115
TYR A:33
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GLU A:57
SER A:220
Interactions
van der Waals
Conventional Hydrogen Bond

b

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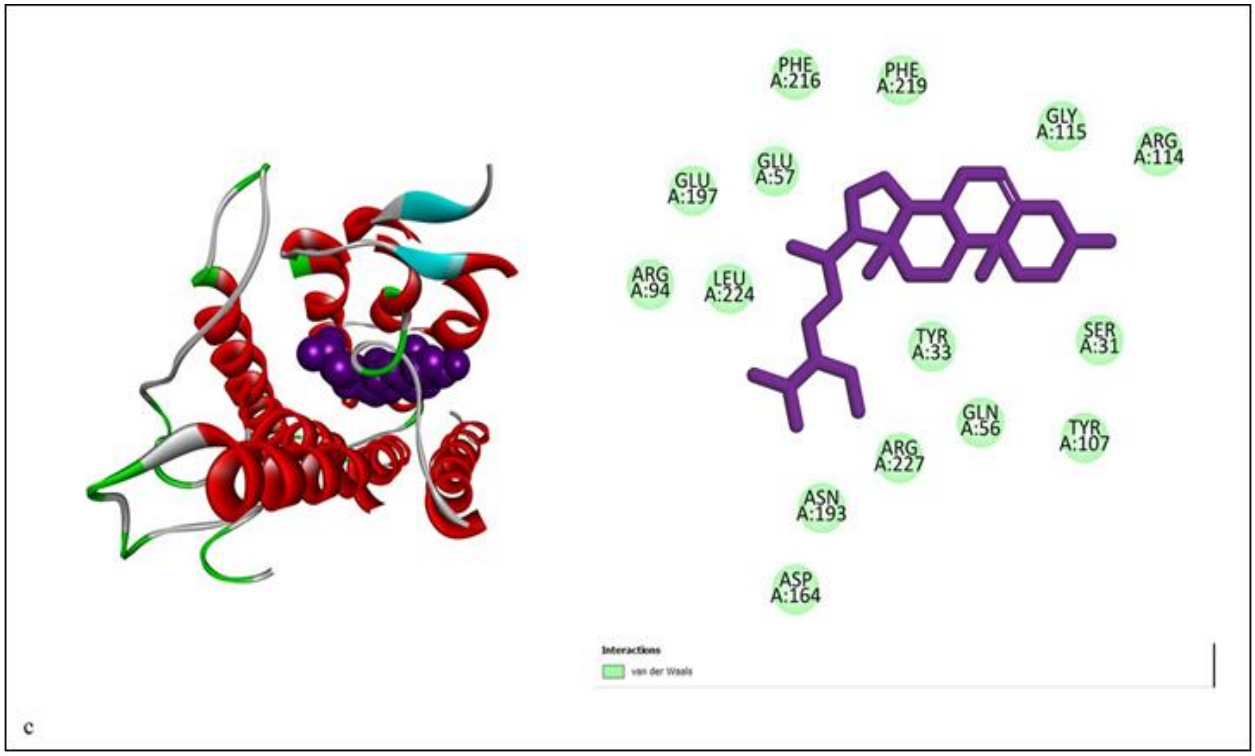

Fig 2 2D and 3D Representation of Interaction between Compounds and Alpha Reductase Enzyme (a) Beta-Cryptoxanthine (b) Beta-Sitosterol (c) Linoleic Acid

➢ *ADME/T Analysis of Selected Top Compounds*

Pharmacological and pharmacokinetic profiling holds significant importance within drug development, enabling the anticipation of a drug's effectiveness. This encompasses factors such as its absorbability, bioavailability, capacity to reach the target site, metabolism, and safe excretion without substantial side effects. Compound's drug-likeness is assessed based on various criteria. Computational tools are widely employed in pharmaceuticals to evaluate a compound's ADME/T attributes, thereby aiding in the selection of prime candidates. The remarkable aspect of the selected drug compounds lies in their adherence to the Lipinski rule, characterized by low molecular weight, topological polar surface area (TPSA), LogS, and xLogP3 values (detailed in Table 4). Moreover, pivotal attributes for therapeutic agent development encompass polar surface area, hydrogen bond donors and acceptors. These models provide valuable insights into predicting absorption, the influence of formulation on permeability, understanding permeability mechanisms, and the likelihood of transporter-mediated drug interactions. Among the chosen ligands, three stood out due to their favorable docking scores and alignment with Lipinski's rule for drug-likeness [18].

Table 4 MW refers to Molecular Weight; LogS predicts aqueous solubility; xLogP3 predicts lipophilicity; Accept H estimates the number of hydrogen bonds the solute accepts from water molecules in an aqueous solution; Donor H estimates the number of hydrogen bonds the solute donates to water molecules in an aqueous solution; TPSA stands for Topological Polar Surface Area, which is a molecular descriptor for drug transport properties.

Ligand name	MW (g/mol)	LogS	xLogP3	Accept H	Donor H	TPSA (Å)
Beta-cryptoxanthine	552	-7.33	12.25	1	1	20.33
Beta-sitosterol	414	-7.90	9.34	1	1	20.23
Linoleic acid	280	-5.05	6.98	2	1	37.30

➢ *Evaluation of Biological Activity*

The PASS webserver was employed to confirm the predicted biological impacts. This assessment revealed that compounds falling within the series 1–3 range possess attributes conducive to combating alopecia. The Pa values, spanning from 0.361 to 0.550 for treating alopecia and 0.056 to 0.376 for inhibiting alpha reductase, suggest the potential for these effects. When the Pa value exceeds the Pi value, it suggests a likely occurrence of the specified biological activity. The condensed findings are outlined in table 5.

Table 5 Forecasting the Biological Activity of Compounds Involves Assessing their Likelihood of being Active (Pa) or Inactive (Pi)

Compound name	Biological activity	Pa	Pi
Beta-sitosterol (1)	Alopecia treatment	0.378	0.123
	Alpha reductase inhibition	0.376	0.003
Beta-cryptoxanthine (2)	Alopecia treatment	0.361	0.137
	Alpha reductase inhibition	0.125	0.082
Linoleic acid (3)	Alopecia treatment	0.550	0.033
	Alpha reductase inhibition	0.056	0.018

IV. CONCLUSION

The condition of androgenetic alopecia (AGA), characterized by hair loss, has a detrimental impact on the psychological and psychosocial well-being of those affected. Non-surgical remedies for AGA predominantly encompass the utilization of finasteride and minoxidil. However, due to the severe adverse effects associated with finasteride (a DHT blocker), individuals grappling with AGA often exhibit reluctance towards its usage. Conventional remedies with purported DHT-blocking properties, including pumpkin seed, have gained attention. In this study, we conducted in silico analysis on the active compounds inherent to pumpkin seed in their interaction with 5-alpha reductase, aiming to predict their potential in blocking DHT. Beyond exhibiting favorable docking scores, these compounds also showcase noteworthy ADME/T properties, indicative of their promising potential. The insights gleaned from this study serve as a foundation for potential in vitro and in vivo validation studies. These subsequent investigations possess the potential to yield a novel drug candidate that offers a safer therapeutic avenue with reduced side effects for AGA treatment. The active phytochemical constituents derived from pumpkin seed exhibited commendable docking scores and remarkable ADME/T attributes. Furthermore, all three compound exhibit biological activity of alopecia treatment and alpha reductase inhibition. The information amassed through this in silico exploration holds the potential to steer future in vivo and in vitro investigations, ultimately advancing validation efforts. In conclusion, the selected compounds display promise as potential therapeutic agents, opening avenues for novel interventions to treat and forestall AGA.

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REFERENCES

[1]. Dhami L. Psychology of Hair Loss Patients and Importance of Counseling. Indian J Plast Surg. 2021 Dec 31;54(4):411-415. doi: 10.1055/s-0041-1741037. PMID: 34984078; PMCID: PMC8719979.

[2]. Sadick NS, Callender VD, Kircik LH, Kogan S. New Insight Into the Pathophysiology of Hair Loss Trigger a Paradigm Shift in the Treatment Approach. J Drugs Dermatol. 2017 Nov 01;16(11):s135-s140.

[3]. Marchetti PM, Barth JH. Clinical biochemistry of dihydrotestosterone. Ann Clin Biochem. 2013 Mar;50(Pt 2):95-107.

[4]. Ustuner ET. Cause of androgenic alopecia: crux of the matter. Plast Reconstr Surg Glob Open. 2013 Nov 7;1(7):e64. doi: 10.1097/GOX.0000000000000005. PMID: 25289259; PMCID: PMC4174066.

[5]. Arca E, Açikgöz G, Taştan HB, Köse O, Kurumlu Z. An open, randomized, comparative study of oral finasteride and 5% topical minoxidil in male androgenetic alopecia. Dermatology. 2004;209(2):117-25. doi: 10.1159/000079595. PMID: 15316165.

[6]. Chandrashekar BS, Nandhini T, Vasanth V, Sriram R, Navale S. Topical minoxidil fortified with finasteride: An account of maintenance of hair density after replacing oral finasteride. Indian Dermatol Online J. 2015 Jan-Feb;6(1):17-20. doi: 10.4103/2229-5178.148925. PMID: 25657911; PMCID: PMC4314881.

[7]. Ganzer CA, Jacobs AR. Emotional Consequences of Finasteride: Fool's Gold. Am J Mens Health. 2018 Jan;12(1):90-95. doi: 10.1177/1557988316631624. Epub 2016 Feb 11. PMID: 26868914; PMCID: PMC5734544.

[8]. Cho, Y. H., Lee, S. Y., Jeong, D. W., Choi, E. J., Kim, Y. J., Lee, J. G., Yi, Y. H., & Cha, H. S. (2014). Effect of pumpkin seed oil on hair growth in men with androgenetic alopecia: a randomized, double-blind, placebo-controlled trial. Evidence-based complementary and alternative medicine : eCAM, 2014, 549721. https://doi.org/10.1155/2014/549721

[9]. Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading.

[10]. Dallakyan, Sargis & Olson, Arthur. (2015). Small-Molecule Library Screening by Docking with PyRx. Methods in molecular biology (Clifton, N.J.). 1263. 243-250. 10.1007/978-1-4939-2269-7_19.

[11]. The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.

[12]. Laskowski R A, Swindells M B (2011). LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J. Chem. Inf. Model., 51, 2778-2786.

[13]. Daina, A., Michielin, O. & Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7, 42717 (2017). https://doi.org/10.1038/srep42717

[14]. N M O'Boyle, M Banck, C A James, C Morley, T Vandermeersch, and G R Hutchison. "Open Babel: An open chemical toolbox." J. Cheminf. (2011), 3, 33. DOI:10.1186/1758-2946-3-33
[15]. Daina, A., Michielin, O. & Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7, 42717 (2017). https://doi.org/10.1038/srep42717
[16]. Filimonov D.A., Lagunin A.A., Gloriozova T.A., Rudik A.V., Druzhilovskii D.S., Pogodin P.V., Poroikov V.V. (2014). Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chemistry of Heterocyclic Compounds, 50 (3), 444-457.
[17]. Biovia, D.S. (2021) Discovery Studio Modeling Environment. Dassault Syst. Release, San Diego, 4.
[18]. Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). In vitro models for selection of development candidatesexperimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev, 23(1), 3-25