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RESEARCH ARTICLE

Unscrewing the DHT-Blocking Properties of *Allium cepa* Extract: Virtual Screening of Active Compounds Against 5-Alpha Reductase and Evaluation of ADME/T Properties of Compounds

Mudassir Alam*, Kashif Abbas

ABSTRACT

Androgenetic alopecia (AGA) occurs in genetically prone men and women and is defined by pattern-related, non-scarring hair follicle shrinkage. It is estimated that up to 80% of men and 50% of women will be affected by AGA at some stage in their lives. The underlying pathophysiology may be traced back to the enzyme 5-alpha-reductase, which is responsible for the conversion of testosterone to dihydrotestosterone (DHT), a more powerful androgen, and its accumulation in hair follicles leads to hair loss. The therapeutic approach for treating AGA mainly relies on the inhibition of 5-alpha-reductase. *Allium cepa* (onion) extract is in trend as a natural remedy for the treatment of AGA. The study aims at in-silico and ADME/T analysis of active compounds present in onion extract against 5-alpha-reductase to evaluate and visualize protein-ligand interaction.

Keywords: Androgenetic alopecia, DHT, Finasteride, Onion extract, 5-alpha reductase.

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INTRODUCTION

Androgenetic alopecia (AGA) is a common condition that affects both men and women. More than just a social concept, hair loss has become a significant aspect of one's self-identity or "body image." Body image is a psychological term that relates to one's thoughts, emotions, perceptions, and behavioral changes connected to one's physical appearance. Despite the fact that baldness is widespread, it often causes psychological disturbance and anguish. Hair thinning and perceived hair loss significantly influence an individual's psyche.[1] The pathophysiology of AGA involves elevated dihydrotestosterone and 5-alpha-reductase level, respectively.[2] The 5 alpha-reductase enzyme transforms testosterone to DHT in target peripheral tissues.[3] DHT binds to androgen receptors in vulnerable hair follicles and activates genes involved in follicular miniaturization.[4] Commonly prescribed drugs for control and treatment of AGA are mainly minoxidil and finasteride. Minoxidil (available both in topical and oral tablet form) is a vasodilator, while finasteride (oral tablet) act as an inhibitor of 5-alpha-reductase thus lowering DHT level.[5] However, people suffering from AGA are reluctant to take finasteride due to its side effects, such as decreased libido, sexual dysfunction, and gynecomastia.[6] Finasteride users, which inhibits dihydrotestosterone formation, suffer substantial physical and mental side effects known as post-finasteride syndrome. Psychiatric diseases and personality characteristics, particularly neuroticism, have an impact on emotional well-being.[7] Due to the severe side effects of finasteride and the patient's reluctance towards the drug, there is a need to find an alternative DHT-blocker to minimize the side effects. *Allium cepa* (onion) extract is a traditional hair growth remedy. Studies have shown that it possesses DHT-blocking properties.[8] Out of all the chemical constituents present in onion extract, the most predominant ones are onionin A and quercetin. Onionin A and quercetin belong to the sulfoxide and flavonoid chemical class,

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respectively.[9] The study aims to evaluate and predict the interaction between the onion extract compounds and the target protein, 5-alpha-reductase.

MATERIAL AND METHODS

In-silico based study was performed using Hewlett Packard laptop with hardware configuration 8 GB RAM, Intel i3 11th generation. The molecular docking tool used for the *in-silico* study was AutoDock Vina tool[10] in PyRx[11]. PyMOL 2.4.1[12] was used for the purpose of protein preparation and visualization of the docked ligand with protein. Ligplot+[13] was used to analyze protein-ligand interaction. For the purpose of ADME/T studies, SwissADME web server[14] was used.

Retrieval and preparation of ligand

The active chemical compounds present in extract of the *A. cepa* were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and NP-MRD database (https://np-mrd.org/) in Structural Data Format (SDF). Compounds used for *In-silico* analysis are mentioned in Table 1. 2D structure of the ligand is given in Figure 1. Ligand preparation was done using PyRx software. The energy of the ligands was minimized using Open Babel tool[15] within the PyRx software and force field used for energy minimization was MMFF94. Finally, all the ligands were converted to AutoDock pdbqt format.

Preparation of the target protein

The three-dimensional structure of 5-alpha-reductase in high resolution was retrieved from the protein data bank (PDB) database (https://www.rcsb.org/) with PDB ID- 7BW1. For the purpose of protein preparation, water molecules were removed to eliminate its hindrance in protein-ligand docking and polar hydrogen were added using PyMOL software. Furthermore, finasteride molecules bound to it were removed to clear its binding site. Finally, the PDB format of protein was converted to pdbqt format for executing AutoDock Vina.

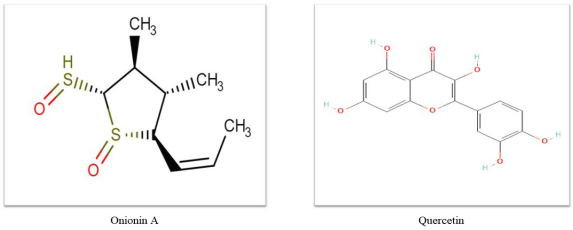


Figure 1: 2D structure of onionin A and quercetin

Virtual screening of the ligands

AutoDock Vina tool within PyRx software was used to virtual screen the retrieved active compounds against the desired target protein. Blind docking was performed with grid box dimensions (43.50 Å ×39.01 Å ×60.70 Å) and centre (-33.23, 13.08, 29.71). The exhaustiveness was set to 8 by default. After completing the screening analysis, the candidates with good docking scores were visualized using PyMOL.

Protein-ligand interactions

Ligplot + was used to make Ligplot for the prediction of hydrophobic and hydrogen bond interaction between the ligand and the target protein.

ADME/T analysis

The ADME/T analysis provides information regarding the tested ligand or chemical compound's absorption, distribution, metabolism, excretion, and toxicity. The analysis is done to knock out undesired compounds with insignificant drug-likeness in order to make the study feasible and time-friendly. The canonical SMILES of the retrieved compounds or hits were used to analyse the pharmacological and physiochemical profile of the hits with the help of SwissADME webserver (http://www.swissadme.ch/). The parameters considered for pharmacological validation were molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, topological polar surface area (TPSA) value and LogS value.

RESULT AND DISCUSSION

Virtual screening result Analysis

The three-dimensional crystal structure of 5-alpha-reductase (PDB ID: 7BW1) was retrieved from protein data bank for docking analysis. The main active compounds of *A. cepa* extract were retrieved from the PubChem and NP-MRD database in Structural Data Format (SDF). The ligands or active compounds were screened against the desired target 5-alpha-reductase using the AutoDock Vina tool of PyRx. The docking score of the top two compounds is mentioned in Table 2.

Table 1: Active compounds present in the onion extract with their compound ID and chemical properties

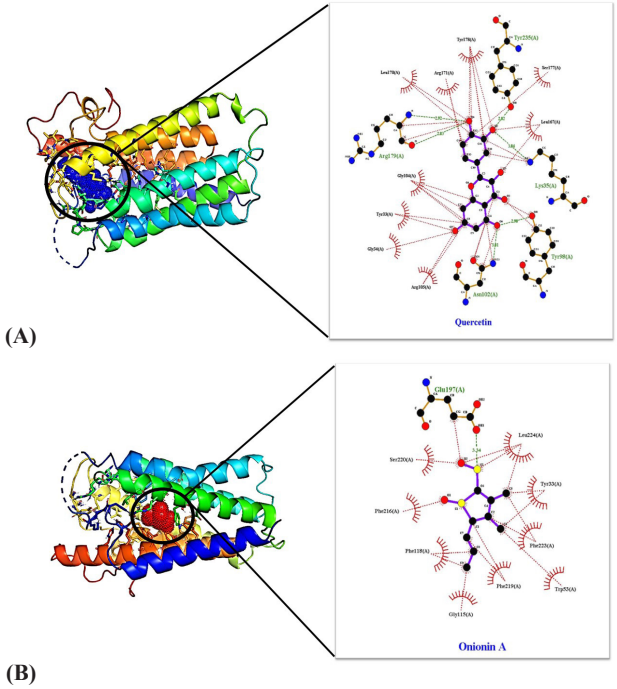
S. NO.	Ligand name	Compound ID	Molecular weight (g/mol)	Molecular formula	Compound class
1	Onionin A	442813 (NP-MRD)	220.35	$C_9H_{16}O_2S_2$	Sulfoxide
2	Quercetin	5280343 (Pubchem)	302.23	$C_{15}H_{10}O_7$	Polyphenolic flavonoid

Table 2: Docking score of the compounds obtained after virtual screening

S. NO.	Ligand name	Compound ID	Molecular weight (g/mol)	Molecular formula	Docking score
1	Onionin A	442813 (NP-MRD)	220.35	$C_9H_{16}O_2S_2$	-6.1
2	Quercetin	5280343 (Pubchem)	302.23	$C_{15}H_{10}O_7$	-9.6

Protein-ligand interaction analysis

Ligplot+ software for protein-ligand has been used for analysis and highlighted the hydrogen bonds and hydrophobic interaction between the protein's amino acids and the ligand. The analysis aid in predicting the binding affinity between the ligand and the target protein. Different types of interactions exhibited by the selected compounds is given in Table 3. The schematic 2D representation of the interaction between the blood-brain permeant ligands and the target protein is given in Figure 2.



(A)

(B)

Figure 2: The grey dotted line depicts the hydrophobic interaction of ligand with a respective amino acid of the target protein while the green dotted line shows the hydrogen bonding of the ligand with the amino acid of the target protein, 5-alpha reductase (A) Onion in A interaction with target protein, 5-alpha reductase (B) Quercetin interaction with target protein, 5-alpha reductase

ADME/T analysis of selected top compounds

Pharmacological and pharmacokinetic profiling is considered an important step in drug development as it helps predict the efficacy of drug such as its absorbability, bioavailability, ability to reach site of action, metabolism and finally, its excretion that too without posing significant side effects. Several factors are taken into consideration to determine a compound's drug-likeness. Computational programs are used widely in pharmaceuticals to test the ADME/T of a compound which aid in selecting top candidates. The striking aspect of the selected drug compound is their low molecular weight, topological polar surface area (TPSA), LogS and Logo/w value satisfy the Lipinski rule (Table 4). The graphical representation of the ADME/T properties of the compounds are given in Figure 2. Furthermore, polar surface area, H-bond donors and acceptors are key characteristics for therapeutic agent development. All of these models

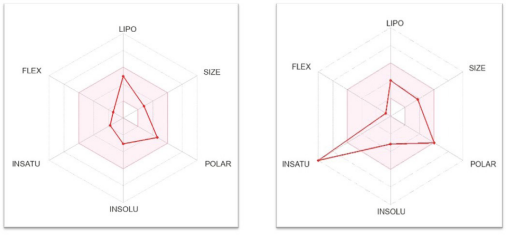


Figure 3: The compound chosen are in the colored zone which predict their suitable physiochemical space for oral bioavailability and exhibit the INSATU (Instauration), LIPO (Lipophilicity), POLAR (Polarity), SIZE (Molecular Weight), INSOLU (Insolubility), and FLIX (Rotatable bond flexibility) parameters.

Table 3: Hydrophobic and hydrogen bond interaction between the compound and active site of the target protein

S. NO.	Ligand name	Hydrophobic interaction	Hydrogen bond interaction
1	Onionin A	Tyr33, Trp53, Gly115, Phe118, Phe216, Phe219, Ser220, Leu224	Gly197
2	Quercetin	Tyr33, Gly34, Arg 105, Leu 167, Leu170, Arg171, Ser177, Tyr178	Lys35, Tyr98, Asn 102, Arg179, Tyr235

Table 4: MW: Molecular Weight; LogS: Predicted aqueous solubility; Log0/w: Predicted Lipophilicity; Accept H: Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution; Donor H: Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution; TPSA: Topological polar surface area, molecular descriptor for drug transport properties such as GI absorption

Ligand name	MW (g/mol)	LogS	Logo/w	Accept H	Donor H	TPSA (Å)	GI absorption
Onionin A	220.35	-3.05	3.14	2	0	92.15	HIGH
Quercetin	302.23	-3.16	1.54	7	5	130.32	HIGH

unscrew the qualitative prediction of absorbability, the impacts of formulation on drug permeability, determining the mechanism(s) of permeability, and the probability for transporter-mediated drug-drug interactions. The two ligands which were chosen on the basis of good docking score and having drug likeness properties by Lipinski's rule.[16]

Androgenetic alopecia (AGA) hair loss condition harms the sufferer's psychological and psychosocial state. The non-surgical treatment for AGA relies on use of finasteride and minoxidil. But people facing AGA are reluctant to use finasteride (DHT blocker) due to its severe side effects. Various traditional remedies are thought to have DHT-blocking properties, and onion extract is one of them. We have performed *In silico* analysis of the active compounds present in onion extract against 5-alpha reductase in order to predict its DHT-blocking efficacy. Apart from good docking score, ADME/T properties also comes out to be remarkable. The data from this study could be used for further *in-vitro* and *in-vivo* validation. Further studies might give a safe novel drug candidate with less side effect for the treatment of AGA.

CONCLUSION

The active phytochemicals present in *onion* extract are rich in flavonoids and have shown a remarkable docking score and ADME/T properties. The data from this *in-silico* study can be used for *in-vivo* and *in-vitro* studies for further validation. We conclude that selected compounds may be a potential candidates for novel therapeutic agents to treat and prevent AGA.

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