

Unscrewing DHT-Blocking Properties of Allium Cepa Extract: Virtual Screening of active Compounds against 5-Alpha Reductase and Evaluation of Adme/T Properties of Compounds

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Abstract:- Androgenetic alopecia (AGA) is a condition that affects both men and women with a genetic predisposition, causing a non-scarring reduction in the size of hair follicles. It is estimated that approximately 80% of men and 50% of women will experience AGA at some point in their lives. The underlying cause can be attributed to the enzyme 5-alpha-reductase, which converts testosterone into dihydrotestosterone (DHT). DHT, a potent androgen formed from testosterone by the action of 5-alpha-reductase enzymes, attaches to androgen receptors in susceptible hair follicles, leading to a decrease in size and eventually halting hair growth. The accumulation of DHT in hair follicles results in hair loss. The primary therapeutic approach for treating AGA revolves around inhibiting 5-alpha-reductase. Recently, there has been growing interest in the use of Allium cepa (onion) extract as a natural remedy for AGA. This study focuses on conducting in silico and ADME/T analyses of the active compounds found in onion extract to assess and visualize their interaction with 5-alpha-reductase protein.

Keywords:- Androgenetic Alopecia, DHT, Finasteride, Onion Extract, 5-Alpha Reductase.

I. INTRODUCTION

Androgenetic alopecia (AGA) is a commonly occurring condition affecting both males and females. Beyond being a societal notion, hair loss has become a significant element of an individual's self-identity or "body image." Body image, a psychological term, encompasses thoughts, emotions, perceptions, and behavioral changes related to one's physical appearance. Despite its prevalence, baldness often leads to psychological distress and suffering. The thinning of hair and the perception of hair loss have a profound negative impact on a person's psyche [1]. The underlying mechanisms of AGA involve elevated levels of dihydrotestosterone and 5-alpha-reductase [2]. The 5-alpha-reductase enzyme converts testosterone to DHT in specific peripheral tissues [3]. DHT binds to androgen receptors in susceptible hair follicles, activating genes involved in

follicular miniaturization [4]. Minoxidil and finasteride are commonly prescribed medications for controlling and treating AGA. Minoxidil is available in both topical and oral tablet forms and acts as a vasodilator, while finasteride, an oral tablet, inhibits 5-alpha-reductase, thereby reducing DHT levels [5]. However, individuals with AGA are hesitant to take finasteride due to its side effects, including reduced libido, sexual dysfunction, and gynecomastia [6]. Users of finasteride, which hampers dihydrotestosterone formation, experience significant physical and mental side effects known as post-finasteride syndrome. Emotional well-being is influenced by psychiatric disorders and personality traits, particularly neuroticism [7]. Considering the severe side effects associated with finasteride and patients' reluctance to use the drug, there is a need to discover alternative DHT blockers to minimize these side effects. Onion extract (Allium cepa) has been traditionally used as a remedy for promoting hair growth. Research has shown that it possesses properties that block DHT [8]. Among the various chemical constituents present in onion extract, the most predominant ones are onionin A and quercetin, belonging to the sulfoxide and flavonoid classes of chemicals, respectively [9]. The aim of this study is to assess and predict the interaction between the compounds found in onion extract and the target protein, 5-alpha-reductase.

➤ Material and Methods

An in-silico study was conducted utilizing an Hewlett Packard laptop equipped with 8 GB RAM and an Intel i3 11th generation processor. The AutoDock Vina tool [10] integrated in PyRx [11] was employed for molecular docking analysis. To prepare the protein and visualize the interaction between the docked ligand and the protein, PyMOL 2.4.1 [12] software was utilized. Ligplot+ [13] was employed for the examination of protein-ligand interactions. For ADME/T (absorption, distribution, metabolism, excretion, and toxicity) studies, the SwissADME webserver [14] was utilized.

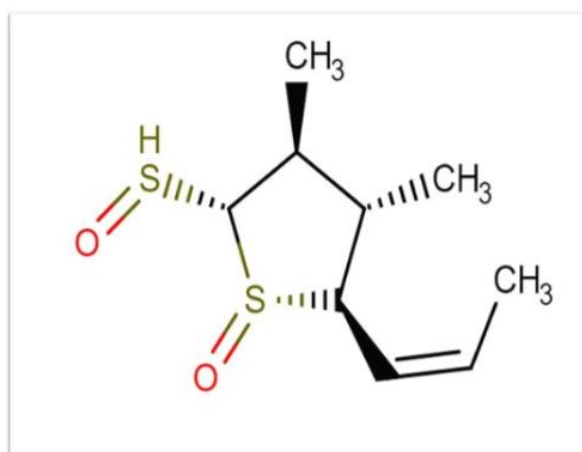
➤ Retrieval and Preparation of Ligands

The chemical constituents with active properties found in the *Allium cepa* extract were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and the NP-MRD database (<https://np-mrd.org/>) in Structural Data Format (SDF). The compounds utilized for the in-silico analysis are listed in Table 1. The 2D structure of the ligand

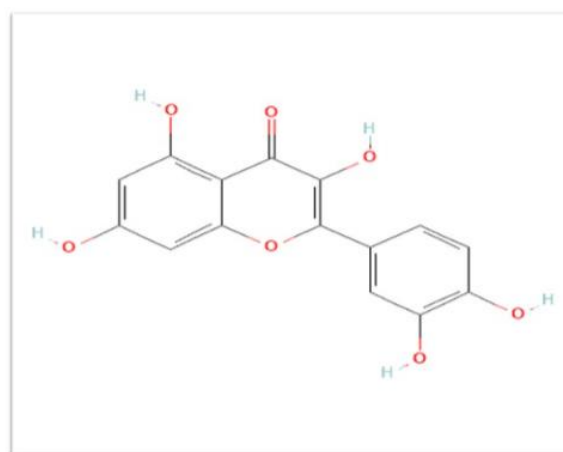
can be observed in Figure 1. Ligand preparation was carried out using PyRx software. The ligands' energy was minimized using the Open Babel tool [15] integrated within the PyRx software, utilizing the MMFF94 force field for energy minimization. Finally, all ligands were converted to AutoDock pdbqt format.

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 ₉ H ₁₆ O ₂ S ₂	Sulfoxide
2	Quercetin	5280343 (Pubchem)	302.23	C ₁₅ H ₁₀ O ₇	Polyphenolic flavonoid



Onionin A



Quercetin

Fig 1 2D Structure of Onionin A and Quercetin

➤ Preparation of Target Protein

The high-resolution three-dimensional structure of 5-alpha-reductase was obtained from the Protein Data Bank (PDB) database (<https://www.rcsb.org/>) with the PDB ID 7BW1. To prepare the protein for subsequent analyses, water molecules were eliminated to prevent interference during protein-ligand docking, and polar hydrogen atoms were added using PyMOL software. Additionally, the finasteride molecule bound to the protein was removed to create an unoccupied binding site. Finally, the protein structure in PDB format was converted to pdbqt format in order to facilitate the utilization of AutoDock Vina.

➤ Virtual Screening of the Ligands

The virtual screening of the active compounds obtained was carried out using the AutoDock Vina tool integrated within the PyRx software. Blind docking was performed with a grid box size of (43.50 Å × 39.01 Å × 60.70 Å) and a center at coordinates (-33.23, 13.08, 29.71). The exhaustiveness parameter was set to the default value of 8. Following the screening analysis, the compounds displaying favorable docking scores were visualized using PyMOL.

➤ Protein-Ligand Interactions

Ligplot+ software was utilized to generate Ligplots, which aided in predicting the hydrophobic and hydrogen bond interactions between the ligand and the target protein.

➤ ADME/T Analysis

ADME/T analysis plays a crucial role in assessing the absorption, distribution, metabolism, excretion, and toxicity of tested ligands or chemical compounds. This analysis helps in eliminating undesirable compounds that lack significant drug-like properties, thereby optimizing the study's efficiency and saving time. To evaluate the pharmacological and physicochemical profiles of the retrieved compounds or hits, the canonical SMILES notation of the compounds was used. This analysis was conducted using the SwissADME webserver (<http://www.swissadme.ch/>). Several parameters were considered for pharmacological validation, including molecular weight, the number of hydrogen bond acceptors and donors, topological polar surface area (TPSA) value, and LogS value.

II. RESULTS

➤ Virtual Screening Result Analysis

The docking analysis utilized the three-dimensional crystal structure of 5-alpha-reductase (PDB ID: 7BW1) obtained from the Protein Data Bank. The primary active compounds found in the extract of *Allium cepa* were

retrieved from the PubChem and NP-MRD databases in Structural Data Format (SDF). The ligands or active compounds were subjected to screening against the desired

target protein, 5-alpha-reductase, using the AutoDock Vina tool integrated within PyRx. The docking scores of the top two compounds are provided in Table 2.

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 ₉ H ₁₆ O ₂ S ₂	-6.1
2	Quercetin	5280343 (Pubchem)	302.23	C ₁₅ H ₁₀ O ₇	-9.6

➤ Protein-Ligand Interaction Analysis

The analysis of protein-ligand interactions was conducted using Ligplot+ software, which facilitated the identification of hydrogen bonds and hydrophobic interactions between the amino acids of the protein and the ligand. This analysis played a crucial role in predicting the

binding affinity between the ligand and the target protein. Table 3 provides information on the various types of interactions displayed by the selected compounds. Additionally, Figure 2 presents a schematic 2D representation illustrating the interactions between the blood-brain permeant ligands and the target protein.

Table 3 Hydrophobic and Hydrogen Bond interaction Between 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

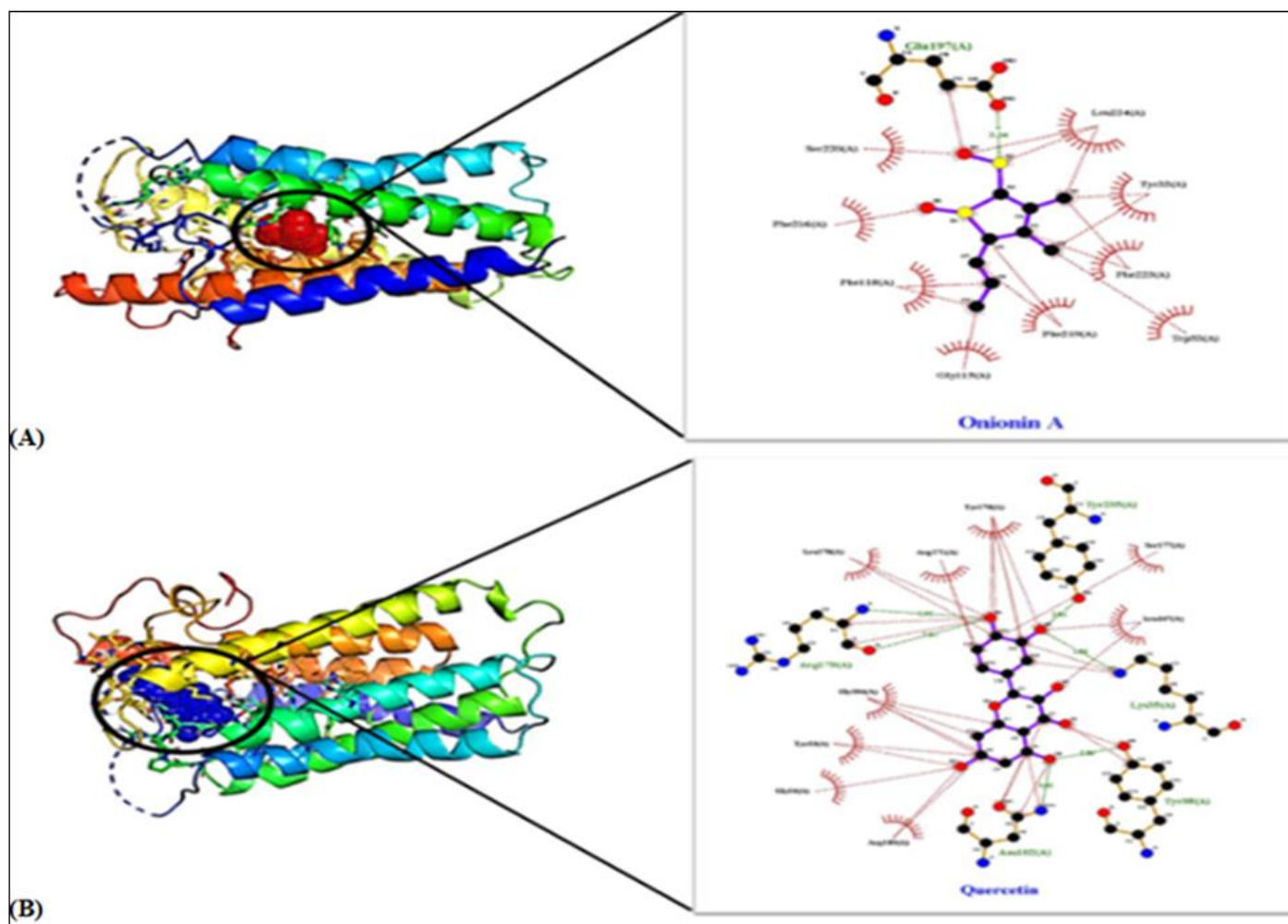


Fig 2 In the schematic representation, hydrophobic interactions between the ligand and the respective amino acids of the target protein are indicated by grey dotted lines. The hydrogen bonding between the ligand and the amino acids of the target protein, 5-alpha reductase, is represented by green dotted lines. Specifically, (A) demonstrates the interaction of Onionin A with the target protein, 5-alpha reductase, while (B) illustrates the interaction of Quercetin with the target protein, 5-alpha reductase.

➤ ADME/T Analysis of Selected Top Compounds

Pharmacological and pharmacokinetic profiling plays a crucial role in drug development as it enables the prediction of a drug's efficacy, including its absorbability, bioavailability, ability to reach the site of action, metabolism, and excretion, while minimizing potential side effects. Several factors are taken into account to assess a compound's drug likeness. Computational programs are widely used in the pharmaceutical field to evaluate the ADME/T (absorption, distribution, metabolism, excretion, and toxicity) properties of a compound, aiding in the selection of top candidate compounds. Notably, the selected drug compounds exhibit desirable characteristics such as

low molecular weight, topological polar surface area (TPSA), LogS, and Logo/w values that meet the Lipinski rule, as indicated in Table 4. The ADME/T properties of the compounds are graphically represented in Figure 2. Additionally, polar surface area, H-bond donors, and acceptors are essential characteristics for therapeutic agent development. These models facilitate the qualitative prediction of absorbability, the impact of formulation on drug permeability, determination of permeability mechanisms, and the likelihood of transporter-mediated drug-drug interactions. The two ligands were chosen based on their favorable docking scores and adherence to Lipinski's rule for drug likeness [16].

Table 4 ADME/T Analysis of Selected Top Compounds

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

Table 4 MW (Molecular Weight) refers to the mass of a molecule, which is an important factor in determining its properties and behavior. LogS predicts the aqueous solubility of a compound, indicating how readily it dissolves in water. LogO/w predicts the lipophilicity of a compound, providing insights into its affinity for lipid environments. Accept H estimates the number of hydrogen bonds that the solute would accept from water molecules in an aqueous

solution, indicating its potential for forming interactions. Donor H estimates the number of hydrogen bonds that the solute would donate to water molecules in an aqueous solution, indicating its ability to participate in hydrogen bonding. TPSA (Topological Polar Surface Area) is a molecular descriptor used to assess drug transport properties, such as gastrointestinal (GI) absorption.

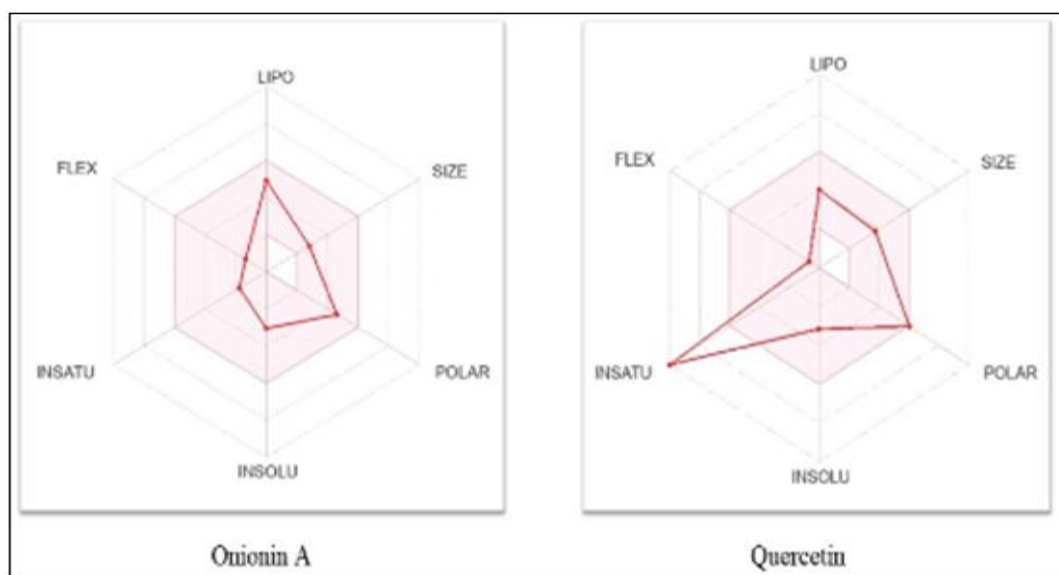


Fig 3 These compounds demonstrate suitable values for parameters such as INSATU (Instauration), LIPO (Lipophilicity), POLAR (Polarity), SIZE (Molecular Weight), INSOLU (Insolubility), and FLIX (Rotatable bond flexibility). These parameters play a crucial role in assessing the suitability of a compound for oral administration and are indicative of its potential pharmaceutical properties

III. DISCUSSION

Androgenetic alopecia (AGA) hair loss condition poses detrimental effect on the sufferer 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. There are various traditional remedies

which are thought to have DHT blocking properties and onion extract is one of them. To predict the efficacy of blocking dihydrotestosterone (DHT), we conducted in-silico analysis of the active compounds found in onion extract against 5-alpha reductase. This analysis aimed to assess the ability of these compounds to inhibit the activity of 5-alpha reductase, thereby potentially reducing DHT levels. In our investigation, we have conducted an in-depth analysis of the

active phytochemicals found in onion extract, revealing their abundant flavonoid content. These phytochemicals have exhibited an impressive docking score, indicating a strong potential for interaction with target molecules. Moreover, out to be remarkable. The data from this study could be used for further *In vitro* and *In vivo* validation. The further studies might give a safe novel drug candidate with less side effect for treatment of AGA.

IV. CONCLUSION

The onion extract contains active phytochemicals that are abundant in flavonoids, demonstrating significant docking scores and favorable ADME/T properties. These findings establish a strong basis for conducting further validation through *in vivo* and *in vitro* studies. The data obtained from this *in-silico* study provide valuable insights and can guide future experimental investigations to confirm the potential of these phytochemicals. By conducting these additional investigations, we aim to confirm the efficacy and safety of the selected compounds. Our research suggests that the identified compounds derived from onion extract hold promise as potential therapeutic agents in the treatment and prevention of androgenetic alopecia (AGA). These compounds possess remarkable attributes that make them attractive candidates for the development of novel interventions for this condition. By employing rigorous scientific methodologies and employing advanced computational analyses, we have gained valuable insights into the potential applications of onion extract in addressing AGA. This groundbreaking research paves the way for future studies and offers a compelling avenue for the development of innovative and effective treatments. Based on our analysis, we conclude that the selected compounds have the potential to serve as promising candidates for novel therapeutic agents in the treatment and prevention of androgenetic alopecia (AGA). These compounds show favorable characteristics and exhibit promising interactions with the target protein, suggesting their potential efficacy in addressing AGA-related concerns. Further research and experimental studies are warranted to validate their effectiveness and explore their clinical application in AGA treatment.

➤ Disclosure statement

The authors state that they have no conflicts of interest to disclose.

➤ Authorship contribution

- M.A. Data collection, analysis and processing.
- K.A. Writing and execution of *in silico* programs.
- T.R. and A.H. Literature search.

➤ Financial Disclosure

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their absorption, distribution, metabolism, and excretion (ADME) properties have been found to be favorable. Apart from good docking score, ADME/T properties also comes

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