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SHORT COMMUNICATION

In Silico Screening of a Phytochemical Naringin, Isolated from *Citrus decumana* var. *paradisi* Against the Genes of Polycystic Ovary Syndrome

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Abstract

Polycystic Ovary Syndrome (PCOS) is a complex hormonal and metabolic disorder. The overproduction of androgens is the primary feature of PCOS. The currently available pharmacological agents recommended for the treatment of PCOS are linked with several adverse effects. Therefore, herbal-based drugs with lesser side effects, have become a favourable trend among people. A flavonoid glycoside, naringin isolated from different fruit parts of *Citrus decumana* var. *paradisi* (Macfad.) H.H.A. Nicholls exhibits a wide range of therapeutic properties. In the traditional system of medicine, it is used to improve ovarian health. To set down scientific evidence, molecular docking analysis was performed to find out the binding affinity of compound naringin with the protein CYP 17-cytochrome P 450, attributing to hyperandrogenism due to its overexpression, leading to PCOS. The docking score values were compared with the standard drug metformin to interpret the effectiveness of flavonoid naringin in the treatment of PCOS.

Keywords: *Citrus decumana* var. *paradisi*, CYP 17, Hyperandrogenism, Molecular Docking, Polycystic Ovary Syndrome

1. Introduction

PCOS is an endocrinological and metabolic disorder prevalent among women of reproductive age with an incidence likelihood of 4 to 20% worldwide[1]. It is characterised by irregular menstrual cycles, hyperinsulinemia, dyslipidemia, oxidative stress, hyperandrogenism and infertility in females. It leads to multiple health complications if remains untreated[2-4]. In women with PCOS ovarian function is disturbed by elevated levels of androgens, prolactin and Luteinizing Hormone (LH) and decreased levels of progesterone. The increased concentration of LH subsequently leads to the production of androgens in the endocrine theca cells of the ovary while the relative Follicle-Stimulating Hormone (FSH) deficiency decreases the ability of granulosa cells of the ovary to convert androgen into estrogen that disturbs follicle maturation and subsequent ovulation[5]. This hormonal imbalance causes the follicles in the ovary to form multiple cysts[6]. High levels of LH can decrease the chance of conception and lead to miscarriage[7].

The current therapeutic interventions include metformin, tamoxifen and clomiphene citrate which are associated with substantial cost and serious side effects such as gastrointestinal symptoms, weight gain and increased insulin resistance[8]. Concerning these side effects of drugs, a new therapeutic approach with lesser side effects, high efficiency and easy availability is desired. This study focuses on the docking affinity of naturally occurring flavonoid naringin against the protein associated with the development of PCOS.

The most understood genetic mechanism of hyperandrogenism in PCOS is as follows:

CYP17 (P450c 17alpha) is a microsomal enzyme expressed in zona recticularis and zona fasciculata

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of the adrenal cortex as well as gonadal tissues and essential for the biosynthesis of testosterone, estradiol and cortisol. Due to overexpression of the CYP17 encoding gene, the activity of CYP17 in adrenal and gonadal sites increases leading to hyperandrogenism in PCOS[9].

Bioinformatics and computational biology have revolutionised the way of drug discovery and drug design. Molecular docking is a budding technique for the screening of potential phytoconstituents against the target protein receptor. The studies revealed the phytochemicals from plants, *Terminalia chebula*, *Terminalia bellirica, Emblica officinalis*[10] and *Angelica sinensis*[11] have been used as potent inhibitors of the CYP17 enzyme analysed through molecular docking.

In the present study the phytoconstituent, naringin, a flavonoid glycoside, extracted from *Citrus decumana* var. *paradisi* [(Macfad.) H.H.A. Nicholls], was taken as a ligand and docked with CYP 17 protein, related to the pathogenesis of PCOS, to find out its binding effectiveness against CYP 17 protein. Additionally, the results obtained were compared with the docking score of known drug metformin against CYP 17 protein. *Citrus decumana* var. *paradisi* (Macfad.) H.H.A. Nicholls, commonly known as grapefruit is a perennial plant belonging to the family Rutaceae. The flavonoid glycoside naringin contributes to the distinct bitterness of citrus fruits and exerts a variety of pharmacological effects such as anti-inflammatory, antioxidant, diuretic, and hypolipidemic[12,13]. Research supports the use of citrus flavonoids as one of the components in Indian traditional medicinal formulations to improve ovarian functions[14]. The previous study conducted by the author reported the isolation and quantification of the compound naringin in methanolic extracts of flavedo, albedo, segment membranes and seeds of grapefruit through HPLC analysis[15]. The Molecular docking analysis of citrus flavonoid, naringin will provide a suitable foundation for the development of novel medication for PCOS.

2. Materials and Methods

2.1 Preparation of Enzyme CYP17

The structure for the enzyme CYP17 (PDB ID: 6WW0) was obtained from the protein data bank[16,17]. The processing of the protein and the ligand was done using Schrodinger Maestro. The pKa corresponding to pH 7.4 was added using the H++ server. A modeller integrated with UCSF Chimera was used for the addition of missing residues and modelling of the loops. A General AMBER Force Field (GAFF) was used to minimize the protein after the structural modelling which was performed using Open Babel[18]. The 3D coordinates for the ligand were obtained from PubChem, and the structure was optimized using ChemAxon Marvin Sketch[19].

2.2 Ligand Preparation

Naringin (4,5,7-trihydroxytrihydroxyflavanone-7-rhamnoglucoside) which served as a ligand consists of 15C atoms in three rings, two of which are benzene rings, connected by three carbon chains and two rhamnose units attached at C7[18] (Figure 1). The 3D structure of the ligand molecule was retrieved from the PubChem database[20,21]. The preparation of the grid box and the parameters were set using Molecular Graphics Laboratory (MGL) tools[22]. The final structure of the protein was then analysed for the potential binding sites using the Castp server[23]. This analysis was done to predict the binding pocket to get an overview of the potential binding sites within the protein.

2.3 Molecular Docking

The molecular docking protocol followed in the procedure was performed using Auto Dock Vina[24], a tool with increased accuracy and enhanced search capacity than Auto Dock 4[25,26]. A rigid docking was performed wherein the ligand atoms were allowed to assume flexibility while keeping the protein atoms rigid. The blind docking was performed to explore the conformational space of the ligand and the results of the blind docking were correlated with the binding

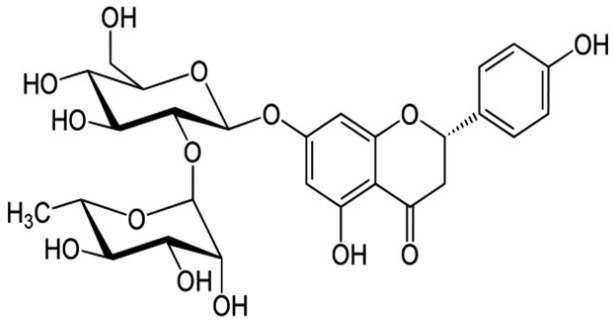


Figure 1. Chemical structure of naringin.

site analysis performed using the Castp server. After analysing the top 10 poses, a docking protocol for the specified binding site was performed. The coordinates size for the grid box size constructed in this case was kept at 28 for x, 33 for y and 31 for z, with an exhaustiveness of 20. The results of the docking were visualised using Schrodinger Maestro[27]. The ligand interaction diagrams were generated using the Protein-Ligand interaction profiler web server[28], a website used for analysing interactions between the ligand and the protein within the binding site. The ligand interaction diagrams were generated using Maestro as well as the Protein-ligand interaction profiler.

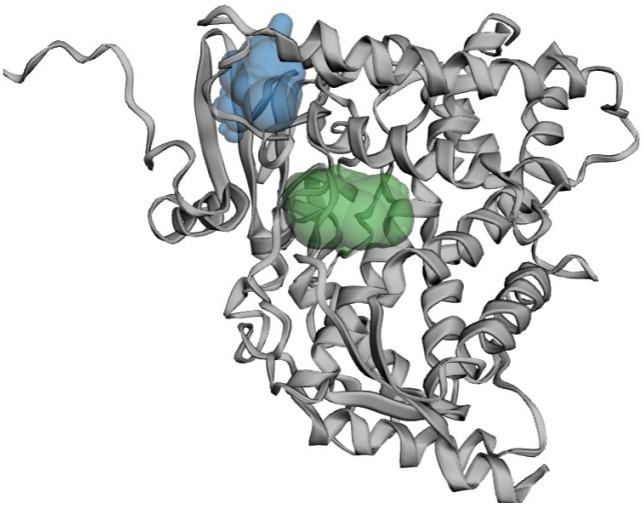
3. Results

The docking protocol was performed using the CYP17A enzyme receptor (PDB ID: 6WW0) and a flavonoid, naringin as the ligand. The docking process was performed using Auto Dock Vina, with the poses ranked according to the binding table affinity calculated. The results presented in Table 1 depict a detailed interaction profile of the ligand-receptor complex, aiding in the stabilisation of the complex. The best pose had a binding affinity of -9.5 Kcal/mol, suggesting a strong interaction with the active residues.

Table 1. The interaction profile of the ligand with active residues of the target protein CYP17

Index	Residue	Amino Acid	Interaction Type
1	216A	ASP	Hydrophobic Interactions
2	219A	PRO	
3	220A	TRP	
4	221A	LEU	
5	221A	LEU	
6	221A	LEU	
7	224A	PHE	
8	45A	ARG	Hydrogen Bonding
9	45A	ARG	
10	101A	THR	
11	208A	ASN	
12	210A	SER	
13	211A	LYS	
14	45A	ARG	Salt Bridge
15	45A	ARG	

The interaction of the small molecule with the target protein is important in defining the stability of the interactions. A set of hydrophobic interactions was found between the backbone of the small molecule and ASP 216, PRO 219, TRP 220, LEU 221 and PHE 224 indicating a strong fit of the molecule within the binding pocket. Hydrogen bonding interactions were found to be between ARG 45, THR 101, ASN 208, SER 210, LYS 211 and the polar functional groups in the molecule. The hydrogen bonds constitute an important polar bonding force that defines most of the stability of the complex. A salt bridge interaction was found between ARG 45 and the molecule. The analysis of the binding volumes for CYP17A helped us to understand the probable binding volumes present in the protein structure. The representation of the surface is shown in Figure 2. The blue surface had a volume of 213.4 $Å^3$, while the green surface was predicted with a volume of 169.5 $Å^3$. These volumes indicate the pockets formed by the protein to incorporate the ligand hence assisting in the binding and exerting a pharmacological response. The interaction diagram of the molecule where the dotted line indicates hydrophobic interaction and a blue solid line indicates hydrogen bonding interactions (Figure 3). The importance of a salt bridge is that it locks the protein region in the confirmation restricting the movement, hence exerting a stabilising effect on the same. The placement of the ligands within the binding sites of the protein aligns with the receptor sites identified and hence an accurate estimate of the



(The blue region shows the largest binding surface, followed by the surface in green).

Figure 2. The surface of the binding sites for CYP17A.

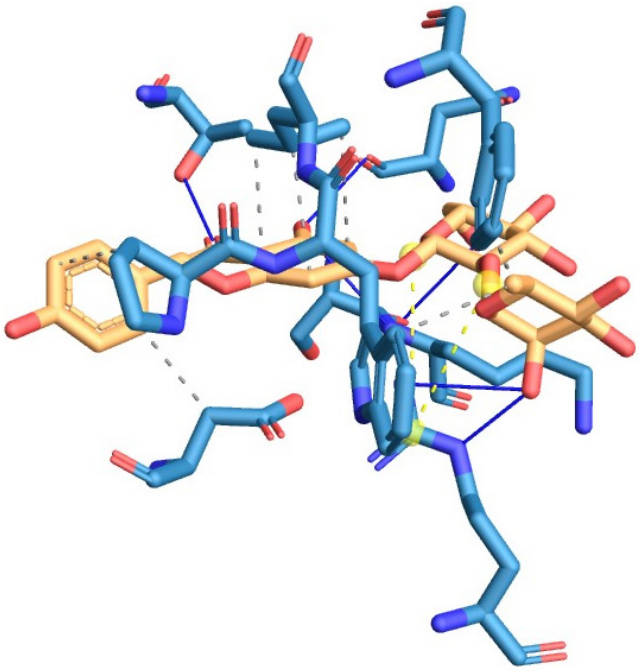


Figure 3. Interaction profile diagram of the docked ligand within the active site of the protein.

binding of the ligand was projected. For comparative analysis, the drug metformin was used as a control whose interaction profile with the target protein CYP 17 is presented in Table 2. The molecular docking for the control drug metformin was also performed using Auto Dock Vina and the binding affinity of the interaction was found to be -5.0Kcal/mol. Metformin showed hydrogen bonding interaction with THR101, ASN208, LEU209 and the terminal amine functionalities. A salt bridge interaction was found to form between ASP 216 and the biguanide backbone. The docked poses of metformin onto the receptor are presented in Figure 4.

The docked poses of naringin into the binding surfaces predicted by the CASTp server are shown in

Table 2. The interaction profile of metformin with active residues of the target protein CYP17

Index	Residue	Amino Acid	Interaction type
1	101A	THE	Hydrogen Bonds
2	208A	ASN	
3	209A	LEU	
4	211A	LYS	
5	216A	ASP	Salt Bridges

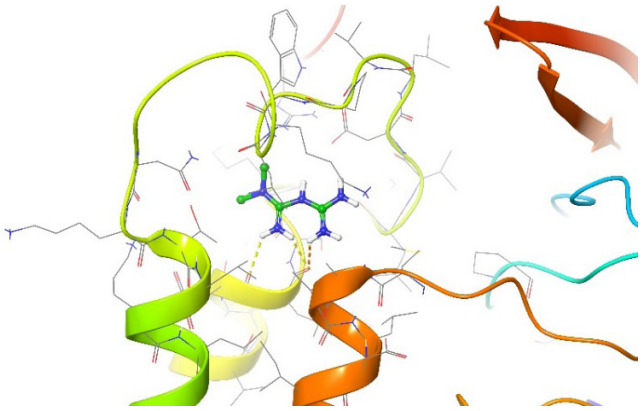


Figure 4. The docked poses of metformin onto the receptor, with a binding affinity of -5.0 kcal/mol.

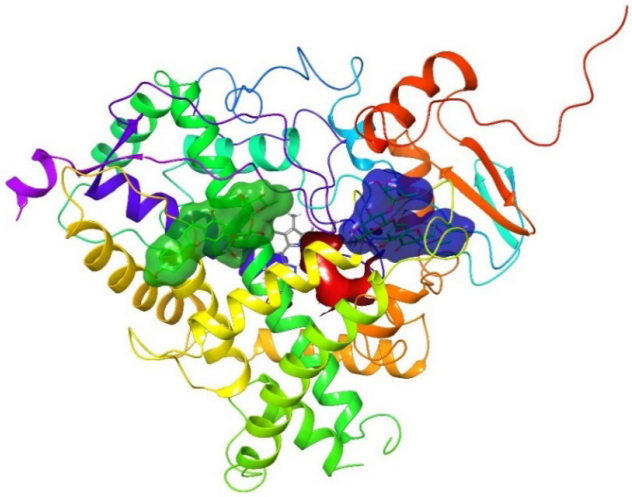


Figure 5. The docked poses of naringin onto the binding surfaces predicted by the CASTp.

Figure 5. The top 2 docked poses of naringin in the blue and green binding pockets

were identified with a binding affinity of -9.5 Kcal/mol and -9.2 Kcal/mol respectively.

This indicates that the volumes predicted by the server and the docking performed using Auto Dock Vina have satisfactory alignment hence reinforcing the role of flavonoid, naringin in the management of PCOS. The interaction profile of the ligand with the active site of protein provides us with a mean to assess the possible bonds the ligand will produce to stabilise it within the binding pocket.

The interaction map displaying the interaction of naringin with CYP17A (Figure 6) has been generated using LigPlot[29,30] further confirming that the inhibitor,

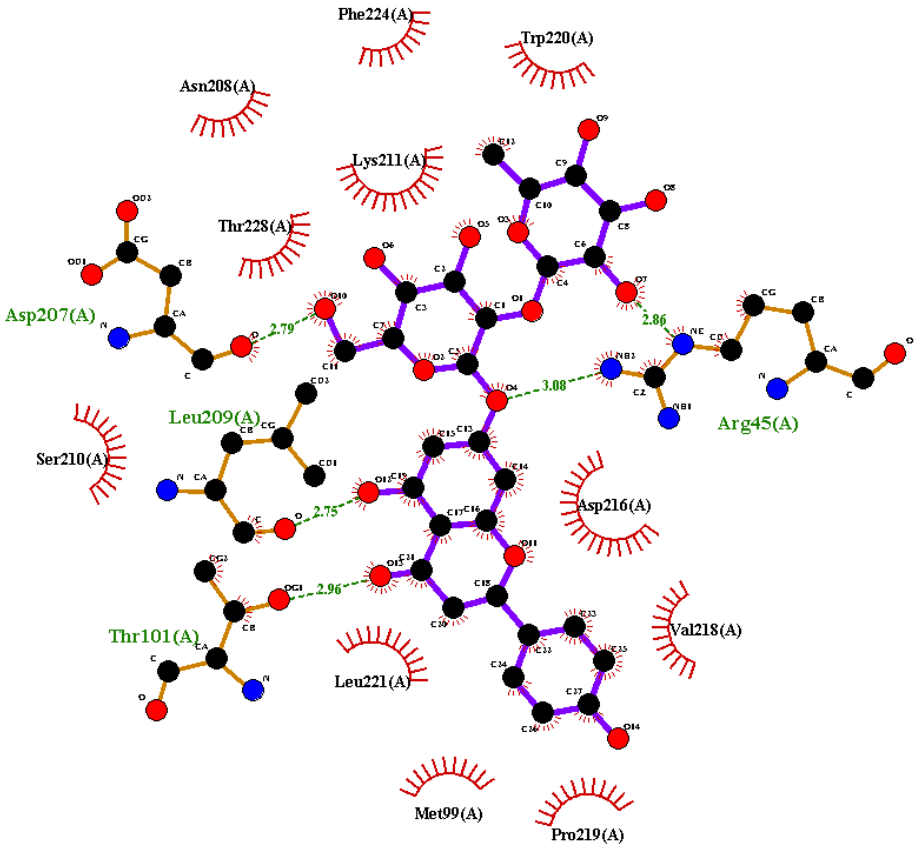


Figure 6. The interaction map generated using LigPlot displays the interaction of naringin with CYP17A, with the bond lengths of interactions.

naringin fits well into the binding pocket of the target protein, hence enabling the tested flavonoid to be employed as a potential candidate in the formulation of herbal drugs against PCOS.

4. Discussion

As oxidative stress, dyslipidemia, hormonal, metabolic and endocrine imbalance are often associated with PCOS, flavonoids due to their diverse biological activities in human health have great therapeutic potential in the treatment of PCOS. It has been investigated that soy isoflavone, a type of flavonoid exerts a beneficial effect on letrozole-induced PCOS in rats which is attributed to their ability to lower testosterone concentration in the peripheral blood[31]. Apigenin, a natural flavonoid from fruits and vegetables could reduce testosterone, estrogen and LH/FSH levels in EV-induced PCOS rats, thus balancing the hormonal levels[32]. Rutin, a flavonoid found abundantly in apricots, grapes, grapefruit plums and oranges can balance lipid profiles in PCOS patients by reducing the expression of adipogenic genes[33]. Various phytoconstituents like Sesamin from *Sesamum indicum* showed a docking energy of -9.23 KJ/mol and -9.14 KJ/mol and lanosterol isolated from *Ficus religiosa* showed a docking energy of -9.37 KJ/mol and -9.63 KJ/mol for the target proteins with PDB ID 3RUK and 1E3K[34].

The more negative value of the docking score displays high docking affinity. The binding affinity of the phytochemical naringin (-9.5 Kcal/Mol) was found to be higher than the control drug (-5.0 Kcal/Mol), confirming that naringin has better binding affinity than metformin hence, proving the effectiveness of the phytochemical against PCOS. These explorations confirm that phytoconstituents owing to their high

docking affinity against target compounds serve as potential agents for the treatment of polycystic ovarian syndrome. Further Structure Activity Relationship (SAR) studies can be used in building a pharmacophore model that will help to analyse the potential pharmacophores with the binding pocket. This information can then be used to make bio-isosteric replacements in the pharmacophore and analyse its binding affinity. A series of derivatives could be designed using computational software and tested on the target.

For infallible outcomes, the molecular docking study requires prudent selection of the target receptor and processing of the receptor for docking. After the protocol design, the analysis, clustering and visualisation of the results are also a task so as not to leave behind a potential pose.

5. Conclusion

A molecular docking system plays a pivotal role in the scientific screening of the drug, saving cost as well as time. The high binding affinity of the compound naringin isolated from *Citrus decumana* var. *paradisi* with the target protein CYP 17 demonstrated the effectiveness of the phytochemical isolated from grapefruit as an important docking agent. The positive findings of naringin in the treatment of PCOS suggest a potential clinical application. The next steps involved performing a preclinical investigation of the molecule, calculating the Absorption, Distribution, Metabolism, Elimination and Toxicity (ADMET) properties and molecular dynamic studies to assess the efficacy as therapeutic and stability of the molecule within the binding pocket with the *in vitro* assessment of the molecule for its safety profiles and subsequent clinical trials on human volunteers.

7. References

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