Phytochemical Screening of Ficus hispida Fruits as a Therapeutic Option against Prostate Cancer Using Pharmacoinformatic and Molecular Dynamic Simulation

MD. Hasanur Rahman[1], Md. Ataur Rahman[1], Md. Sarwar Zahan[2], Abdullah Al Mamun Sohag[3], Silme Islam[2], Riyan Al Islam Reshad[4], Partha Biswas[5], Bablu Gupta[6], Redwanul Islam[6], Md Abdul Hannan[3], Woojin Kim[1], Md Jamal Uddin[2*], Bonglee Kim[1*]

[1]Kyung Hee University, South Korea, [2]ABEx Bio-Research Center, Bangladesh, [3]Bangladesh Agricultural University, Bangladesh, [4]Shahjalal University of Science and Technology, Bangladesh, [5]Jashore University of Science and Technology, Bangladesh, [6]Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Bangladesh

Submitted to Journal:
Frontiers in Oncology

Specialty Section:
Pharmacology of Anti-Cancer Drugs

Article type:
Original Research Article

Manuscript ID:
1008199

Received on:
31 Jul 2022

Journal website link:
www.frontiersin.org

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

M.H.R. designed the project. generating the data, analyzing the data, and wrote the manuscript. M.S.Z. and P.B. analyzing the data and wrote the manuscript. A.A.M.S., R.A.I.R and S.I. wrote the manuscript. B.G. and R.I. collected the data. M.J.U., M.A.H., M.A.R., W.K and B.K. supervised and reviewed the manuscript. All authors read and approved the final manuscript.

Keywords

Ficus hispida, prostate cancer, ADME, molecular dynamics, Anti-cancer therapeutics

Abstract

Word count: 198

Prostate cancer is one of the leading causes of death and most common cancer type in men. Since androgen receptor (AR) plays a critical role in prostate tumor development, it can be the potent target in treating prostate cancer. In this study, the potential of Ficus hispida in treating prostate cancer was determined through inhibiting 5T8E androgen receptor. The phytochemicals from fruit of Ficus hispida were selected for molecular docking and molecular dynamics (MD) simulation to inhibit 5T8E. PASS online and ADMET test was performed to select more specific phytochemicals that are relevant to prostate cancer treatment and has potential as drug. Five phytochemicals named Nodakenetin (CID: 26305), Isowigtheone hydrate (CID: 66728267), Methyl chlorogenate (CID: 6476139), 7-Hydroxycoumarin (CID: 5281426) and Gallic acid (CID: 370) have been identified through the screening process and they have the highest negative binding affinity (kcal/mol) as well as binding free energy (kcal/mol). 100 ns MD simulation confirmed the structural binding stability of the five phytochemicals with the targeted androgen receptor protein. Therefore, this study can conclude that the five Ficus hispida fruit phytochemicals can inhibit the androgen receptor activity in prostate gland and can be developed as a treatment option against prostate cancer.

Contribution to the field

M.H.R. designed the project. generating the data, analyzing the data, and wrote the manuscript. M.S.Z. and P.B. analyzing the data and wrote the manuscript. A.A.M.S., R.A.I.R and S.I. wrote the manuscript. B.G. and R.I. collected the data. M.J.U., M.A.H., M.A.R., W.K and B.K. supervised and reviewed the manuscript. All authors read and approved the final manuscript.

Ethics statements

Studies involving animal subjects

Generated Statement: No animal studies are presented in this manuscript.

Studies involving human subjects

Generated Statement: No human studies are presented in this manuscript.

Inclusion of identifiable human data

Generated Statement: No potentially identifiable human images or data is presented in this study.

Data availability statement

Generated Statement: The data analyzed in this study is subject to the following licenses/restrictions: Data send upon request.. Requests to access these datasets should be directed to Md Jamal Uddin, hasan800920@gmail.com .

Phytochemical Screening of *Ficus hispida* Fruits as a Therapeutic Option against Prostate Cancer Using Pharmacoinformatic and Molecular Dynamic Simulation

MD. Hasanur Rahman[1, 2#], Md. Ataur Rahman[2, 3#], Md. Sarwar Zahan[1], Abdullah Al Mamun Sohag[4], Silme Islam[1], Riyan Al Islam Reshad[1, 5], Partha Bishwas[1,5], Bablu Gupta[6], Redwanul Islam[7], Md. Abdul Hannan[4], Woojin Kim[3], Md Jamal Uddin[1*], Bonglee Kim[2, 3*]

[1]ABEx Bio-Research Center, East Azampur, Dhaka-1230, Bangladesh.

[2]Department of Pathology, College of Korean Medicine, Kyung Hee University, Hoegidong Dongdaemungu, Seoul 02447, South Korea.

[3]Korean Medicine-Based Drug Repositioning Cancer Research Center, College of Korean Medicine, Kyung Hee University, Seoul 02447, South Korea.

[4]Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

[5]Department of Genetic Engineering and Biotechnology, Faculty of Biological Science and Technology, Jashore University of Science and Technology, Jashore, 7408, Bangladesh.

[6]Department of Environmental Science, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj-8100, Bangladesh.

[7]Department of Biotechnology and Genetic Engineering, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj-8100, Bangladesh.

[#]These authors contributed equally to the work.

*** Correspondences:**

Md Jamal Uddin, hasan800920@gmail.com

Bonglee Kim, bongleekim@khu.ac.kr

Abstract

Prostate cancer is one of the leading causes of death and most common cancer type in men. Since androgen receptor (AR) plays a critical role in prostate tumor development, it can be the potent target in treating prostate cancer. In this study, the potential of *Ficus hispida* in treating prostate cancer was determined through inhibiting 5T8E androgen receptor. The phytochemicals from fruit of *Ficus hispida* were selected for molecular docking and molecular dynamics (MD) simulation to inhibit 5T8E. PASS online and ADMET test was performed to select more specific phytochemicals that are relevant to prostate cancer treatment and has potential as drug. Five phytochemicals named Nodakenetin (CID: 26305), Isowigtheone hydrate (CID: 66728267), Methyl chlorogenate (CID: 6476139), 7-Hydroxycoumarin (CID: 5281426) and Gallic acid (CID: 370) have been identified through the screening process and they have the highest negative binding affinity (kcal/mol) as well as binding free energy (kcal/mol). 100 ns MD simulation confirmed the structural binding stability of the five phytochemicals with the targeted androgen receptor protein. Therefore, this study can conclude that the five *Ficus hispida* fruit phytochemicals can inhibit the androgen receptor activity in prostate gland and can be developed as a treatment option against prostate cancer.

Keywords: *Ficus hispida*, Prostate cancer, ADME, Molecular dynamics, Anti-cancer therapeutics.

Introduction

Cancer, a scary disease and current global burden, is related to the uncontrolled growth and division of cells in the body. The causative reason of this terrifying disease is related tothe environmental factors as well as genetic factors which includes inherited germline mutations, altering the DNA methylation rate and microRNA alteration (Hashemi et al.). Among the types of the cancer, breast cancer (2.6 million), lung cancer (2.21 million), colon-rectum (1.93 million), and prostate cancers (1.41 million) are responsible for most of the death (WHO, 2022). In man, the most common cancer is prostate cancer which second most leading cause of death in men and is in continuous with more than 160000 new cases each year in the United States (Litwin and Tan). In terms of nature of the disease, prostate cancer is clinically quite diverse as some patients shows an aggressive disease outcome whereas others exhibit an indolent disease with low tendency to progression. Moreover, the high mortality rate is partly due to the early identification of the disease as well as lack of effective medication for curbing the metastasis. However, the standard treatment of prostate cancer is related to radiotherapy and surgery. On the other hand, the patients who are not suitable for either radiotherapy or surgery are treated with androgen ablation therapy, which effectively shorten the androgen-dependent tumors. But, this treatment is often followed by recurrent androgen-independent prostate cancer with frequent metastases that impacts the quality of life of the patient (Testa et al., Sayegh et al., 2021).

Androgen receptor (AR) is a member of the nuclear steroid hormone receptor family. The AR plays pivotal role in the growth of prostate by regulating transcription, cellular proliferation, and apoptosis. AR also have essential functions for proliferation and survival of prostatic cells; hence they have become a prominent target for the treatment of prostate cancer (Banerjee et al.). Prostate tumor growth is almost dependent upon the androgen receptor pathway and hence therapies aimed at blocking this signaling axis are useful tools in the management of this disease (Bhati et al., 2019). However, bicalutamide and flutamide, anti-androgens drugs, attach to the ligand-binding domain (LBD) of AR, which causes the inhibition of androgen attachment to the LBD. Moreover, enzalutamide, a nonsteroidal antiandrogen, binds with a greater affinity for the LBD of AR. The atrial fibrillation follow-up investigation of rhythm management (AFFIRM) trials confirmed that enzalutamide significantly prolonged the overall survival of patients. However, some prostate cancer patients become resistance to enzalutamide (Iguchi et al., 2019, Tran et al., 2009). So, developing new drug is essential to reduce the mortality of prostate cancer.

Recently, researchers have been identified that dietary phytochemicals might be an innovative solution for restricting cancer due to their safe nature and availability. According to some study, cancer risk is decreased with increasing consumption of vegetables and fruits. Because phytochemicals of vegetables and fruits not only interrupt in the aberrant signaling pathways of cancer but also synergize with chemotherapy and radiotherapy (Pratheeshkumar et al., 2015). In this regard, blocking a specific protein via phytochemical that is often overexpressed in prostate cancer can help prevent or delay the disease from spreading to other parts of the body. *Ficus hispida* has been reported to show a variety of biological properties anticancer. Although, only a few well-designed research on *F. hispida* has investigated the active components that contribute to pharmacological activities (Cheng et al., 2020, Jansen et al., Hurtado-Barroso et al., 2020).

Computational approaches are useful tools for the preliminary work of drug discovery. Different tools have been used to find the association among the ligands and receptor binding site through physics-based equations. These approaches are simple and reliable that helps to speed up the drug discovery process (Brito, 2011). The estimation of absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of probable drug molecules are a pivotal challenge in the process of drug discovery (Bhati et al., 2019). Various bioinformatics tools are developed that helps to predict the ADME/T properties of the ligands. Moreover, molecular docking tools assist to predict interaction between ligand-target at a molecular level (Morris and Lim-Wilby, 2008). Recently, molecular dynamics (MD) simulations have become popular to predict the binding and unbinding kinetics of drugs, since these physico-chemical properties play important role in drug design and optimization. MD simulations also helps in the estimation of the thermodynamics and kinetics related to the drug–target identification and binding pattern (De Vivo et al., 2016). So, in the light of the aforementioned evidence, we selected anticancer phytochemicals *of Ficus hispida* fruit based on their pharmacokinetic properties including ADMET. Then, we find out the best binding ligands (i.e., phytochemicals) based on their anticancer properties by PASS online results and conducted the molecular docking study against AR protein (PDB ID: 5T8E). In addition, to validate the results from the docking study, the ligands were subjected to molecular dynamics simulations using Desmond (Schrödinger Release V 21.2) software.

Materials and Methods

Ligand library generation

Dr. Duke's phytochemical and ethnobotanical database (www.phyto-chem.nal.usda.gov/phytochem/search), as well as reviews and research articles were used to generate a library of the phytochemicals for this study. A total of 119 phytochemicals of *Ficus hispida* were found from these sources. Then, only 13 *Ficus hispida* fruits anti-prostate cancer phytochemicals were chosen for further studies. The selected phytochemicals chemical structures were retrieved from PubChem database at the National Institutes of Health website (Kim S, 2021).

Ligand activity prediction

A popular server named PASS (Prediction of Activity Spectra for Substances), which predicts outcomes based on a substance's structural constitution, that was used to compare the biological activity of 13 phytochemicals (Filimonov D.A., 2014). The Structure Activity Relationship Base is used to predict the likelihood that a specific substance will belong to the active and inactive subsets of that substance (SAR Base). The input was provided in SMILES (Simplified Molecular Input Line Entry System) format for the phytochemicals structure. We calculated the Pa (probable activity) and Pi (probable inactivity) values for each ligand. Therefore, only the activities with Pa > Pi and Pa > 0.5 were taken into consideration (Poroikov et al.).

ADMET studies

In clinical trials, QikProp (Schrödinger Suit 21.2) finds lead compounds that perform better in terms of ADMET (absorption, distribution, metabolism, excretion, and toxicity). QikProp is a powerful ADMET prediction tool for pharmaceutical analysis. We further analyzed the PASS analysis passed 10 bioactive ligand molecules pharmacological properties linked to prostate cancer.

Protein retrieval and preparation

The X-ray crystallographic structure of Androgen receptor protein (PDB ID: 5T8E), was retrieved from the RCSB PDB protein data bank (www.rcsb.org) (H.M. Berman, 2000). To perform better interactions with selected ligands to a targeted protein, the protein crystal structure must prepare well. Therefore, protein crystal structures need to be built before docking so that hydrogen atoms can be added, hydrogen bonds can be optimized, atomic conflicts can be reduced, and various other tasks can be executed that are not part of the x-ray crystal structure refinement process.(Sastry et al.). The protein preparation wizard in Schrödinger suite 21.2 was used to prepare the retrieved 5T8E protein structure . In the protein preparation wizard, assigned bond

orders, CCD database, addition of hydrogens, formation of zero-order bonds to metals, formation of disulfide bonds properties was used. Moreover, to fix the protein missing side chain and loops in the receptor, the missing side chains and loops properties were used by using Prime in Schrödinger suite 21.2 (Schrödinger-Prime, 2021). Also, the fixing of cap termini, the deletion of waters was beyond 5Å, and the generation of heat states pH level was 7.0 +/- 2.0. Epik in Schrödinger suite 21.2 were to calculate these properties (Schrödinger-Epik, 2021). The refine tab and the OPLS3e force field were used to assign the H-bond in PROPKA at pH level 7.0 and restrict the degradation to RMSD 0.30 Å with heavy atom coverage (Schrödinger-Maestro, 2021).

Ligand preparation

The standardized drugs and phytochemicals, three-dimensional (3D) structures were retrieved in SDF format with individual PubChem CID code from the open-source PubChem database (www.pubchem.ncbi.nlm.nih.gov). All the ligand structures were prepared using LigPrep application in Schrödinger suite 21.2 (Schrödinger-LigPrep, 2021). The OPLS3e force field and the Epik ionizer were used to execute minimization at a standard pH range of 7.0 to (+/-) 2.0, with a maximum number of conformers per structure of 32 and a RMSD of 1.0 Å.

Receptor grid generation

The grid restricts the active site to a more condensed region of the receptor protein, and the grid makes easier for the ligand to bind in its desired location (Ban et al.). Initially, the Receptor Grid Generation tool included in Schrodinger suite 21.2 was utilized to construct a grid with a Van der Waals residue using the parameters of scaling factor = 1.0 and partial charge cutoff = 0.25. A cube-shaped enclosure was formed all the way around the active site (reference ligand active site). Following that, the volume of the grid box was modified such that it measured 14 x 14 x 14 Å degrees for the docking process.

Molecular docking

The selected protein-ligand complexes structure was taken from docked post-viewing file for post docking visualization purposes. Further the study of non-bond interactions, hydrophobicity and binding affinities of the complexes were evaluated by Maestro's XP (extra precision) molecular docking application (Schrödinger-Maestro, 2021). The post-docked structures of the receptor-ligand complex were visualized using the Discovery Studio Visualizer 21 (BIOVIA, 2021). To clarify and visualize the non-boned and non-covalent bonded interactions, high binding affinities showed complexes were visualized in Discovery Studio Visualizer 21.

MD simulations

100 ns MD simulations were used to assess the consistency of the potential ligand phytochemicals binding to the targeted receptor active binding site. The thermodynamic stability of receptor-ligand complexes was examined using the Desmond application in Schrödinger suite 21.2 (Schrödinger-Desmond, 2021). Also, A predetermined TIP3P water technique was developed for this framework to maintain a specific volume with an orthorhombic periodic bounding box shape at 10 Å distance. For electrically neutralizing the framework, suitable ions like O+ and 0.15 M NaCl have been selected and dispersed at randomly inside the solvent system. After developing the solvency protein system with a ligand complex, the proposed system was optimized and simplified by applying the Desmond default protocols implemented by force field OPLS3e within the Desmond module (Roos et al., 2019). Moreover, A 50 ps capture periods and an energy of 1.2 ps were utilized with the Nose-Hoover temperature combining technique and isotropic technique NPT. Then the final assemblies were maintained at 300 K and one atmospheric pressure (1.01325 bar). Finally, the results data of the 100 ns molecular dynamic simulation were visualized by using Simulation Trajectory Analysis (SID) application of Schrödinger's suite 21.2 (Schrödinger-Desmond, 2021). SID was used to evaluate the stability of 100 ns runtime trajectory complex structures root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), protein–ligand contacts (P-L contact), ligand-protein contacts (L-P contact), and hydrogen bond interactions.

Results

PASS online predication

Using the PASS online tool, all 13 phytochemicals were assessed for their potential to prevent prostate cancer. 9 out of 13 of the phytochemicals among them passed Pa and Pi value filtering (Table 1). The phytochemicals predicted with more than one feature with Pa > Pi were only taken into consideration based on the combined properties such as anti-metastatic, prostate cancer treatment, and androgen antagonist. Since they have a better possibility of being produced experimentally, the PASS predicted phytochemicals with Pa > 0.5 were taken into consideration. Pa values below 0.5 were eliminated because they lack pharmacological potency. Also, PASS can help to reduce side effects of a molecule even though it cannot predict the binding affinity for new

therapeutic targets. The filtered 10 phytochemicals mentioned above were taken for ADME analysis before being used in docking analysis.

ADME analysis

The ADME properties of a drug candidate are crucial for the regulatory approval process since they assist researchers and drug developers to understand the safety and efficacy of a drug candidate (Kennedy, 1997). In this study the phytochemicals that had been screened using the PASS program were assessed by QikProp for drug-like properties (ADME) investigations. For estimating the ADME properties, variables such as molecular weight, molecular volume, QPlogPo/w, QPlogPw, QPlogS, QPlogKp, QPlogKhsa, QPCaco, and SASA were considered, whereas all the 10 phytochemicals undergone ADME screening and passed Lipinski's filter. The chosen phytochemicals for the study fit within acceptable ranges, according to analysis of ADME qualities, showing their potential as drug-like phytochemicals (Table 2). The ADME passed phytochemicals molecular weights range is, from 154.122 to 456.707. Also, the ADME passed phytochemicals molar volume range is from 502.685 to 1387.897. 3,4-Dihydroxybenzoic acid (CID: 72), Gallic acid (CID: 370), Nodakenetin/(-)-Marmesin (CID: 26305), 7-Dihydroxycoumarin (CID: 5281426), Alpinumisoflavone (CID: 5490139), Methyl chlorogenate/Chlorogenic acid methyl ester (CID: 6476139), 6-[(R)-2-Hydroxy-3-methyl-3-butenyl]-7-hydroxycoumarin (CID: 12050842), (6R,9R) -Roseoside (CID: 44570408), Isowigtheone hydrate (CID: 66728267), and Murrayaculatine (CID: 101416188) demonstrated a complete adherence to Lipinski's rule and was projected by QikProp analysis to have a high proportion of human absorption (Table 2). It is anticipated that phytochemicals that have passed Lipinski's rules and the ADME qualities will make for suitable oral medications (Benet et al.).

Table 1: PASS online prediction results

SL No.	CID/SID	Phytochemicals	Pa	Pi	Activity
1.	72	3,4-Dihydroxybenzoic acid	0,262	0,100	Prostate disorders treatment
			0,383	0,020	Cancer associated disorders treatment
			0,170	0,044	Cancer procoagulant inhibitor
2.	370	Gallic acid	0,183	0,085	Prostate cancer treatment
			0,198	0,160	Prostate disorders treatment
			0,374	0,024	Cancer associated disorders treatment
			0,167	0,045	Cancer procoagulant inhibitor
3.	26305	Nodakenetin / (-)-Marmesin	0,072	0,034	Androgen antagonist
			0,302	0,041	Prostate cancer treatment
			0,284	0,085	Prostate disorders treatment
			0,085	0,028	Androgen antagonist
			0,475	0,013	Prostate cancer treatment
			0,456	0,027	Prostate disorders treatment
5.	5281426	7-Hydroxycoumarin	0,099	0,023	Androgen antagonist
			0,359	0,050	Prostate disorders treatment
			0,283	0,046	Prostate cancer treatment
			0,334	0,055	Cancer associated disorders treatment

6.	5490139	Alpinumisoflavone	0,253	0,055	Prostate cancer treatment
			0,227	0,208	Cancer associated disorders treatment
			0,075	0,032	Androgen antagonist
			0,615	0,012	Prostate disorders treatment
			0,212	0,069	Prostate cancer treatment
8.	6476139	Methyl chlorogenate / Chlorogenic acid methyl ester	0,317	0,037	Prostate cancer treatment
9.	12050842	6-[(R)-2-Hydroxy-3-methyl-3-butenyl]-7-hydroxycoumarin	0,227	0,207	Cancer associated disorders treatment
10.	44570408	(6R,9R)-Roseoside	0,239	0,121	Antineoplastic (pancreatic cancer)
11.	66728267	Isowigtheone hydrate	0,095	0,024	Androgen antagonist
			0,429	0,018	Prostate cancer treatment
12.	101416188	Murrayaculatine	0,370	0,026	Cancer associated disorders treatment
			0,118	0,089	Cancer procoagulant inhibitor
13.	463911958	Benzyl alpha-beta-glucopyranoside	0,340	0,035	Antineoplastic (pancreatic cancer)

Table 2: ADME result

SL NO	CID/SID	Phytochemicals	mol_MW	SASA	Volume	QPlogPw	QPlogPo/w	QPlogS	QPPCaco	QPlogKp	QPlogKhsa	RuleOfFive
1	72	3,4-Dihydroxybenzoic acid	154.122	329.839	502.685	9.936	0.014	-0.779	27.436	-4.61	-0.905	0
2	370	Gallic acid	170.121	340.445	523.341	12.016	-0.585	-0.681	10.027	-5.488	-0.987	0
3	26305	Nodakenetin / (-)-Marmesin	246.262	466.594	789.661	7.69	2.048	-3.094	984.592	-2.672	-0.057	0
4	5281426	7-Hydroxycoumarin	456.707	683.818	1387.897	8.06	6.2	-6.712	342.203	-2.829	1.331	1
5	5490139	Alpinumisoflavone	162.145	344.677	536.547	7.338	0.71	-1.425	624.506	-2.988	-0.512	0
6	6476139	Methyl chlorogenate / Chlorogenic acid methyl ester	336.343	589.7	1026.72	8.613	3.673	-5.174	687.613	-2.371	0.559	0
7	12050842	6-[(R)-2-Hydroxy-3-methyl-3-butenyl]-7-hydroxycoumarin	368.34	643.055	1111.512	18.784	0.045	-3.069	20.553	-5.271	-0.63	0
8	44570408	(6R,9R)-Roseoside	246.262	482.857	817.506	9.861	1.484	-2.608	421.722	-3.033	-0.286	0
9	66728267	Isowigtheone hydrate	356.374	622.184	1098.6	11.292	2.826	-4.396	125.1	-3.586	0.253	0
10	101416188	Murrayaculatine	207.185	358.098	606.273	6.486	-0.919	-0.825	20.622	-5.613	-0.534	0

Molecular docking and virtual screening analysis

Following the screening processes (PASS and ADME property analysis), Glide docking with Extra precision was performed on all the 10 ligand phytochemicals (XP). Every plausible conformation for each low energy conformer in the specified binding site is determined using glide XP model (Friesner et al., 2006). While the protein conformation is fixed, the torsional degrees of each ligand are relaxed during the process. Conversely 7 out of the 10 phytochemicals shows their docking score. The docking scores, Glide energy, eModel value, and the quantity of hydrogen bond forms were utilized to rank the preferred conformation for each ligand during the docking process. Considering the flexibility of both the ligand and the protein, it might be because of the Glide and Prime module that proper binding is depicted. Pose selection was facilitated by the emodel values. Analysis of the Glide energy term, which is the Coulomb's and van der Waal's interaction, can also consider the energy of the ligand interaction. However, we selected best 5 phytochemicals for our further final analysis based on their docking performance.

Table 3: Molecular docking score result

SL NO	PubChem CID	Name of Ligand	Docking score	XP GScore	Glide gscore	Glide emodel
1	12050842	6-[(R)-2-Hydroxy-3-methyl-3-butenyl]-7-hydroxycoumarin	-10.214	-10.217	-10.217	-50.299
2	26305	Nodakenetin	-9.946	-9.946	-9.946	-40.88
3	66728267	Isowigtheone hydrate	-9.199	-9.224	-9.224	86.664
4	6476139	Methyl chlorogenate	-8.683	-8.685	-8.685	115.57
5	5281426	7-Hydroxycoumarin	-7.653	-7.663	-7.663	-37.54
6	370	Gallic acid	-7.457	-7.464	-7.464	-37.052
7	72	3,4-Dihydroxybenzoic acid	-6.771	-6.771	-6.771	-32.924

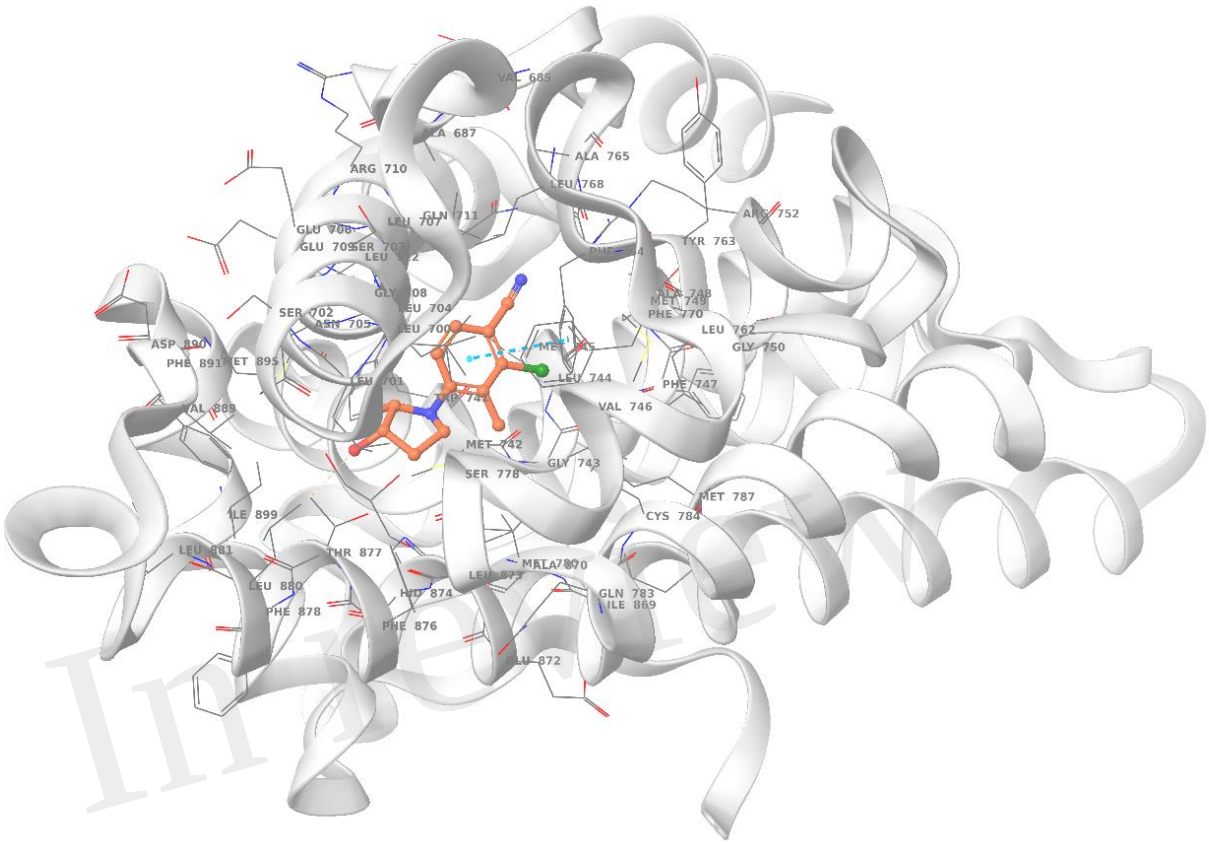

Figure 1: 5T8E androgen receptor structure

Binding interactions through glide XP docking

The selected five ligand interactions with the desired protein were analyzed using BIOVIA Discovery Studio Visualizer 21 tool. The CID: 26305 has been found to generate several conventional and carbon-hydrogen bonds with the targeted AR protein. It was found that two typical hydrogen bonds formed at the positions of Asn705 (3.22 Å) and Arg752 (6.31Å) (Figure 2). A Pi-Sigma and a Pi-Pi bond were also seen during the interaction in the position of Met745 (4.54 Å) and Phe764 (4.72Å), as well as five Pi-Alkyl bonds also found at the positions of Met749 (5.73 Å), Met780 (5.65 Å), Leu701 (5.06 Å), Leu704 (5.22 Å), and Leu880 (5.82 Å). Three conventional hydrogen bonds were discovered in the CID: 66728267 at the positions of Asn705 (3.81 Å), Arg752 (5.83 Å), and Met745 (3.83 Å) (Figure 3). There are five Pi-Alkyl bonds at Met780 (4.42 Å), Phe876 (3.85 Å), and Leu873 (4.04 Å). Additionally, a Pi-Pi link was discovered at Phe764 (5.26 Å). For the phytochemical CID: 6476139, five conventional hydrogen bonds were

discovered to form at the positions of Met745 (3.67 Å), Arg752 (5.8 Å), Phe764 (5.37 Å), Asn705 (3.18 Å), and Thr877 (4.84 Å) position (Figure 4), also three Pi-Alkyl and one Pi-Sulfur bonds have found in this phytochemical. These positions are Leu704 (4.95Å), Leu707 (5.27 Å), Met749 (6.02 Å) and Met742 (4.87 Å). One conventional hydrogen bond, one Pi-Sulfur, one Pi-Alkyl, and one Pi-Pi bond were discovered in the interaction analysis of phytochemical CID: 5281426 at the positions of Leu704 (4.27 Å), Met787 (8.30 Å), Met745 (4.10, 5.41 Å), and Phe764 (5.34 Å) (Figure 5). One conventional hydrogen bond, one Pi-Sulfur bond, and one Pi-Pi link were also discovered in the investigation of CID: 370 phytochemicals at the positions of Leu704 (3.78 Å, 4.03 Å), Met745 (4.32 Å), and Phe764 (5.35 Å) (Figure 6).

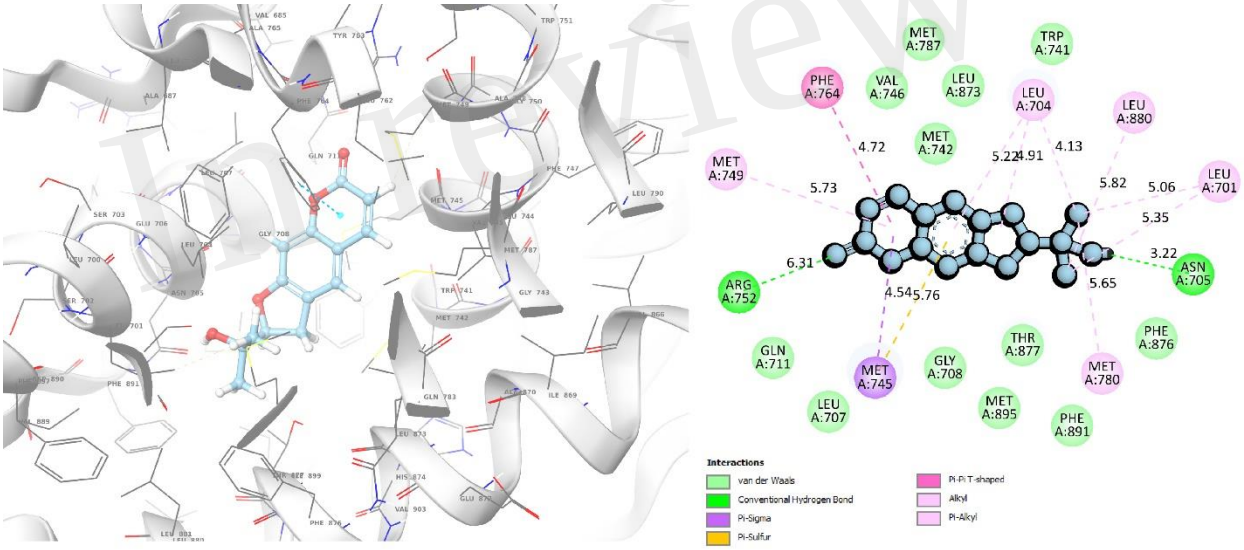

Figure 2: 5T8E and CID: 26305 binding interactions. Left is the 3D interactions and right are the 2D interaction.

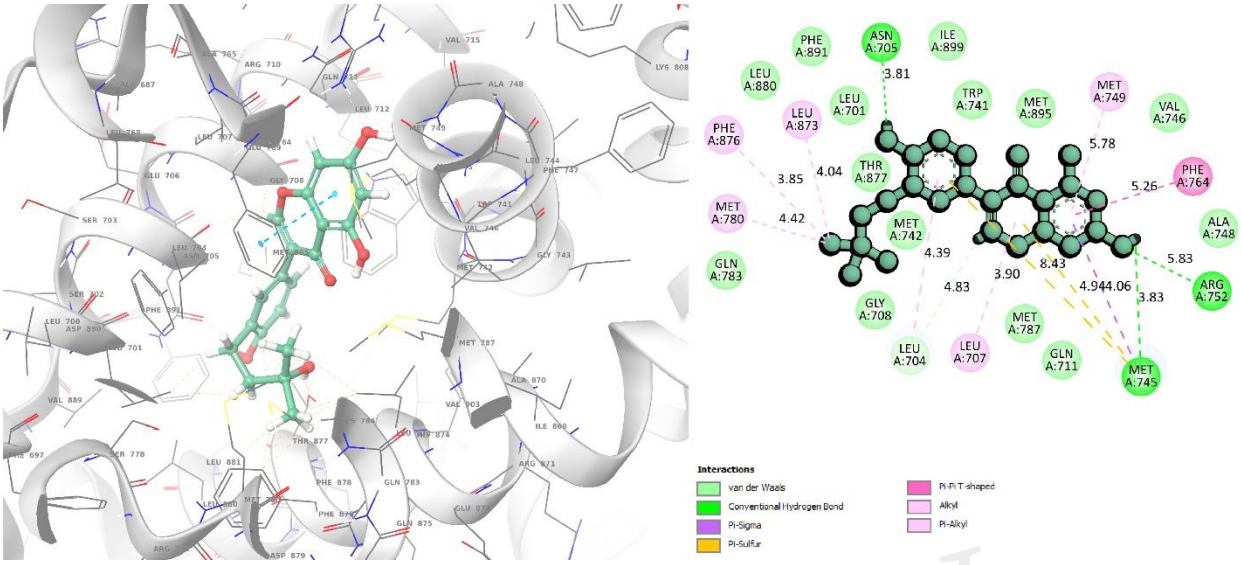

Figure 3: 5T8E and CID: 66728267 binding interactions. Left is the 3D interaction and right is the 2D interaction.

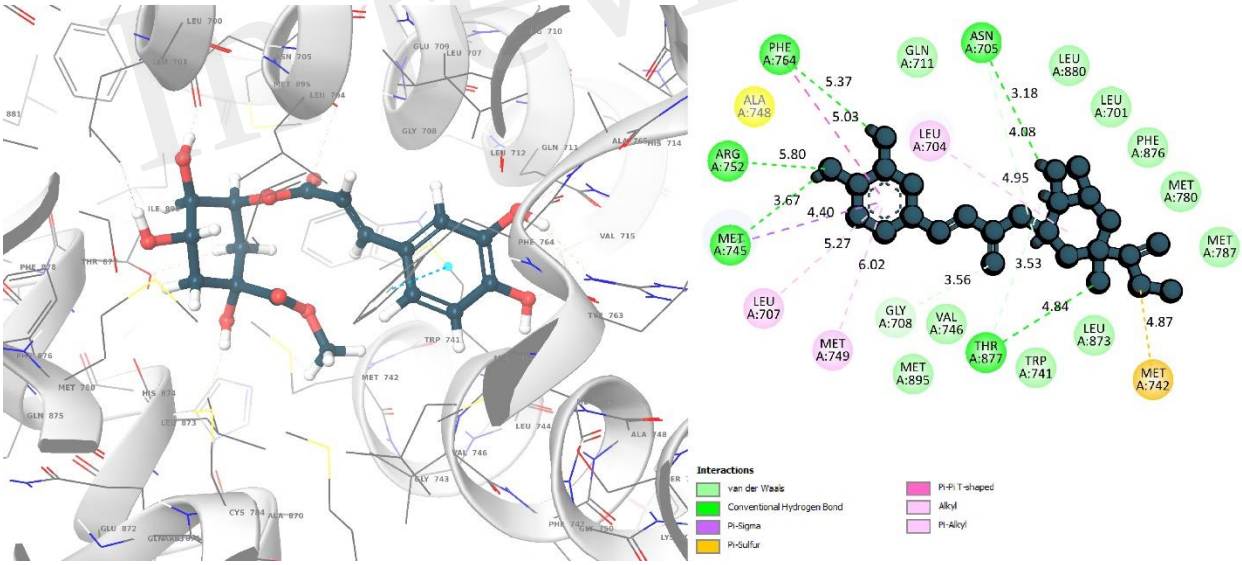

Figure 4: 5T8E and CID: 6476139 binding interactions. Left is the 3D interaction and right is the 2D interaction.

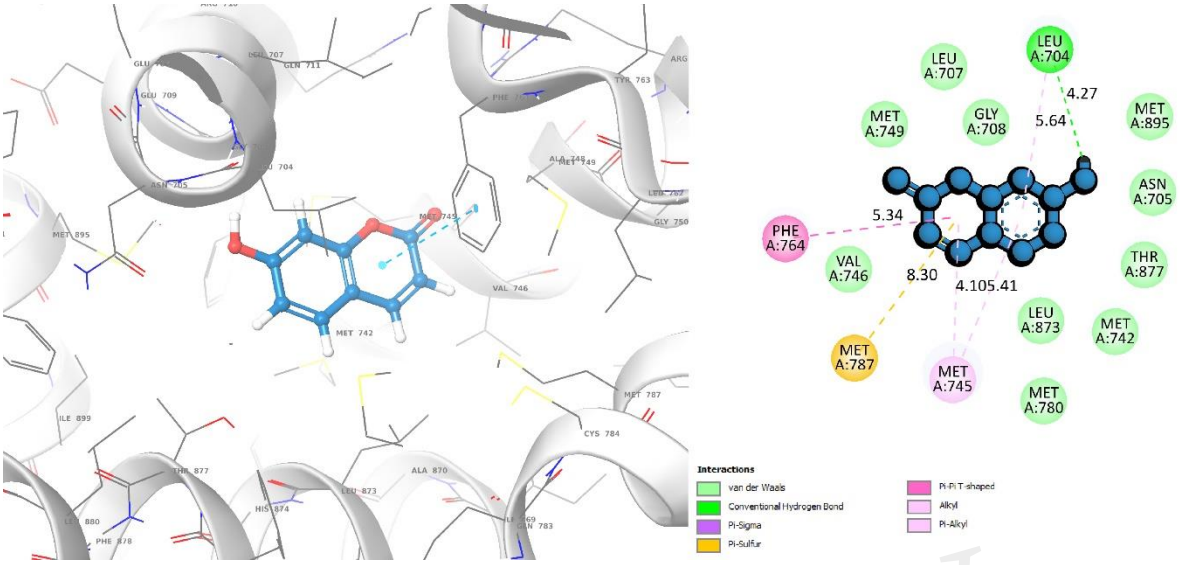

Figure 5: 5T8E and CID: 5281426 binding interactions. Left is the 3D interaction and right are the 2D interaction.

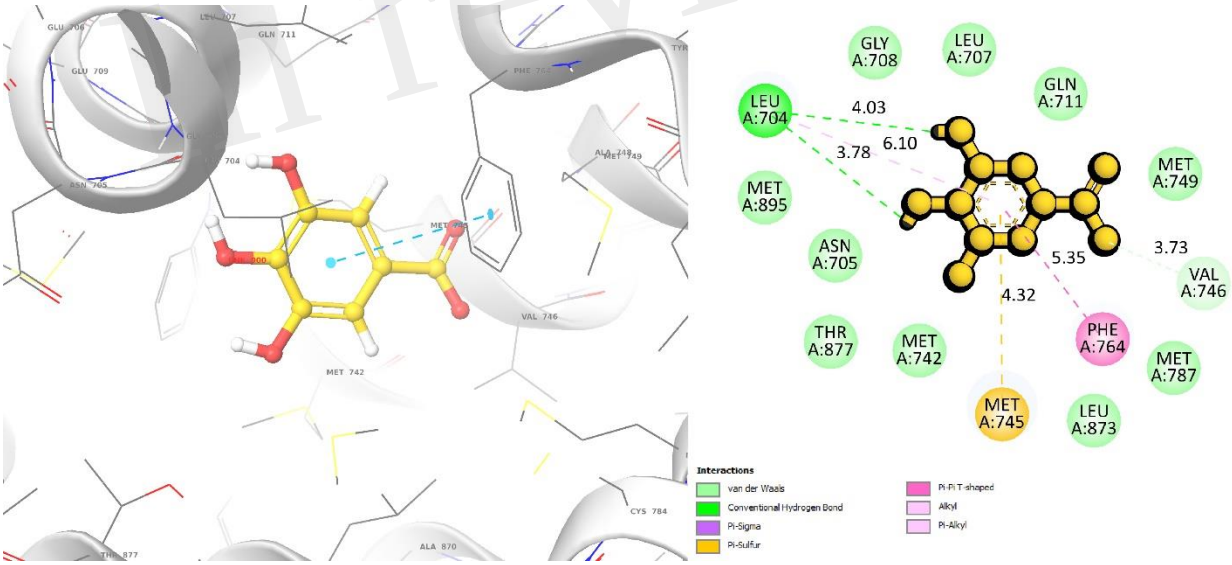

Figure 6: 5T8E and CID: 370 binding interactions. Left is the 3D interactions and right are the 2D interaction.

MD simulation analysis

MD simulations can be used to verify the stability of protein-ligand complexes in an artificial environment. Using MD simulations, one can track the changes in protein conformation over time to better understand how proteins move (Collier et al., 2020). Therefore, we performed 100 ns MD simulations of the protein-ligand complex structures to determine the candidate ligand molecules consistency in binding to the target receptor protein active site. The protein-ligands complex's stability was shown using a food and drug administration (FDA) approved control drug namely

Flutamide (CID: 3397) which also shows -8.52 kcal/mol docking score with the targeted receptor 5T8E. To analysis the MD simulation results, RMSD, RMSF, Rg, SASA, P-L contact, L-P contact, and hydrogen bond interactions were described.

RMSD analysis of protein

The average movement of atoms between two reference frames has been measured by using RMSD. The admissible range of the RMSD according to a reference frame is 1 to 3 Å. The RMSD progression of a protein (left Y-axis) and a ligand are shown in Figure 7 (right Y-axis). After all protein frames are aligned on the reference frame backbone, the RMSD for CID: 26305 (Nodakenetin), CID: 66728267 (Isowigtheone hydrate), CID: 6476139 (Methyl chlorogenate), CID: 5281426 (7-Hydroxycoumarin), CID: 370 (Gallic acid) and the control drug CID: 3397 (flutamide) and the control drug CID: 3397 (flutamide) is calculated (Figure 7). With the reference 5T8E protein backbone, all six phytochemicals performed very identical stable binding within the range of 0.7 - 2.1 Å.

It has been observed that the fluctuation was increasing for all phytochemicals at the beginning of 0-45 ns. After 46 ns, the state of equilibration became apparent, and after 73 ns, the fluctuation started to decrease. Therefore, it should consider how the fluctuations will be optimized over the length of the extensive simulation run and how stable a ligand is regarding to the protein and its binding pocket is shown by its RMSD. The RMSD of the ligand is displayed in the plot after the protein-ligand complex is initially aligned on the reference protein backbone and the RMSD of the ligand heavy atoms is measured. Also, we computed the RMSD of the phytochemicals in that scenario to assess the stability of the phytochemicals in compared to the control drug. In this instance, the binding complexes observations discovered optimal RMSD for all other phytochemicals.

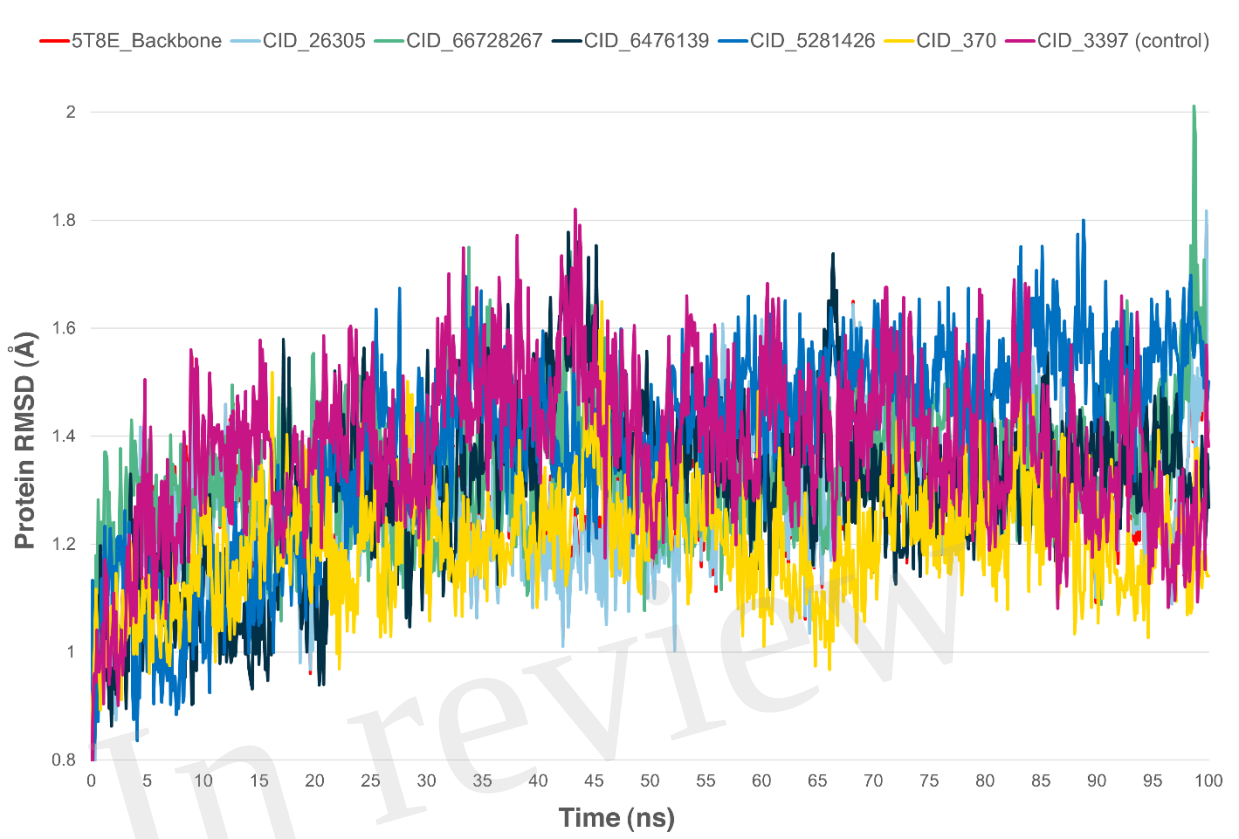

Fig 7: Protein RMSD of CID: 26305 (sky blue), CID: 66728267 (green), CID: 6476139 (navy blue), CID:5281426 (blue), CID: 370 (yellow) and CID: 3397 (violet) to 100 ns simulation time.

RMSD analysis of ligand

To assess the phytochemicals stability relative to the control drug, Ligand RMSD calculations were calculated. Figure 8 shows the computed RMSD for the phytochemicals CID: 26305 (Nodakenetin), CID: 66728267 (Isowigtheone hydrate), CID: 6476139 (Methyl chlorogenate), CID: 5281426 (7-Hydroxycoumarin), CID: 370 (Gallic acid) and the control drug CID: 3397 (flutamide). The complex docking structure was first aligned on the reference protein's backbone to measure the RMSD of the phytochemicals. The phytochemicals can move away from its initial binding site because the observation found the optimum RMSD for two phytochemicals namely CID: 26305 (Nodakenetin), CID: 66728267 (Isowigtheone hydrate), with the exception phytochemical CID: 6476139 (Methyl chlorogenate), CID: 5281426 (7-Hydroxycoumarin), CID: 370 (Gallic acid), whose distance are significantly larger than the RMSD value of control drug CID: 3397 (flutamide). It has been found that the fluctuation for CID: 370 (Gallic acid) started to increase at 0 - 15 ns. After 15 ns, the state of equilibration became apparent, and after 75 ns, the fluctuation started to decrease. It was observed that CID: 5281426 (7-Hydroxycoumarin)

fluctuation was increasing at the start of 0 – 6 ns. After 6 ns, the state of equilibration became apparent, and after 66 ns, the fluctuation started to decrease. For CID: 6476139 (Methyl chlorogenate), it was reported that the fluctuation increased and decreased continuously from the start to the 51 ns, then after 51 ns, the rest simulation time frame showed equilibration.

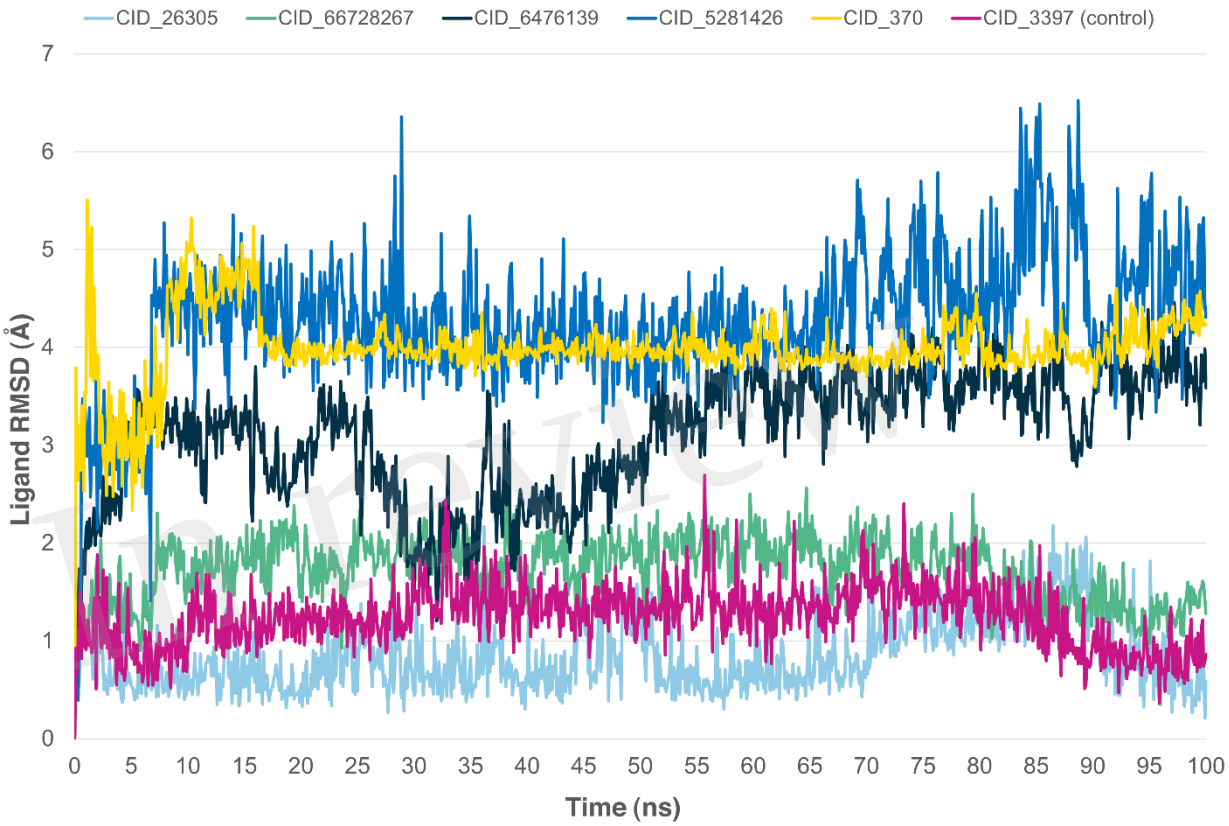

Figure 8: Ligand RMSD of CID: 26305 (sky blue), CID: 66728267 (green), CID: 6476139 (navy blue), CID:5281426 (blue), CID: 370 (yellow) and CID: 3397 (violet) to 100 ns simulation time.

RMSF analysis

The RMSF value is crucial for protein characterization because it gives insight into the local alterations in the protein as well as the protein chain (Bharadwaj et al., 2021). The results are given in Figure 9, CID: 26305 (Nodakenetin) fluctuation in ACE607 4.5 Å, in ASN691 1.7 Å, in ARG846 1.8 Å and in the THR918 2 Å. Also, the main peaks of fluctuations between 0 and 250 residues were maximum. With 2.3 Å in ASN848 and 4.4 Å in NMA919, the control drug CID: 3397 (Flutamide) demonstrated the greatest and most consistent fluctuations between 175 – 250 residual positions. The CID: 66728267 (Isowigtheone hydrate) phytochemical fluctuates at a maximum of 4.3 Å in ACE607 and 2.6 Å in ASN691. The RMSF of CID: 6476139 (Methyl chlorogenate), CID: 5281426 (7-Hydroxycoumarin), and CID: 370 (Gallic acid) had the same flow

as control drug, whereas the RMSF of CID: 26305 (Nodakenetin) protein complex and CID: 6476139 (Methyl chlorogenate) protein complex had higher values for all AA residues in the protein-ligand complex.

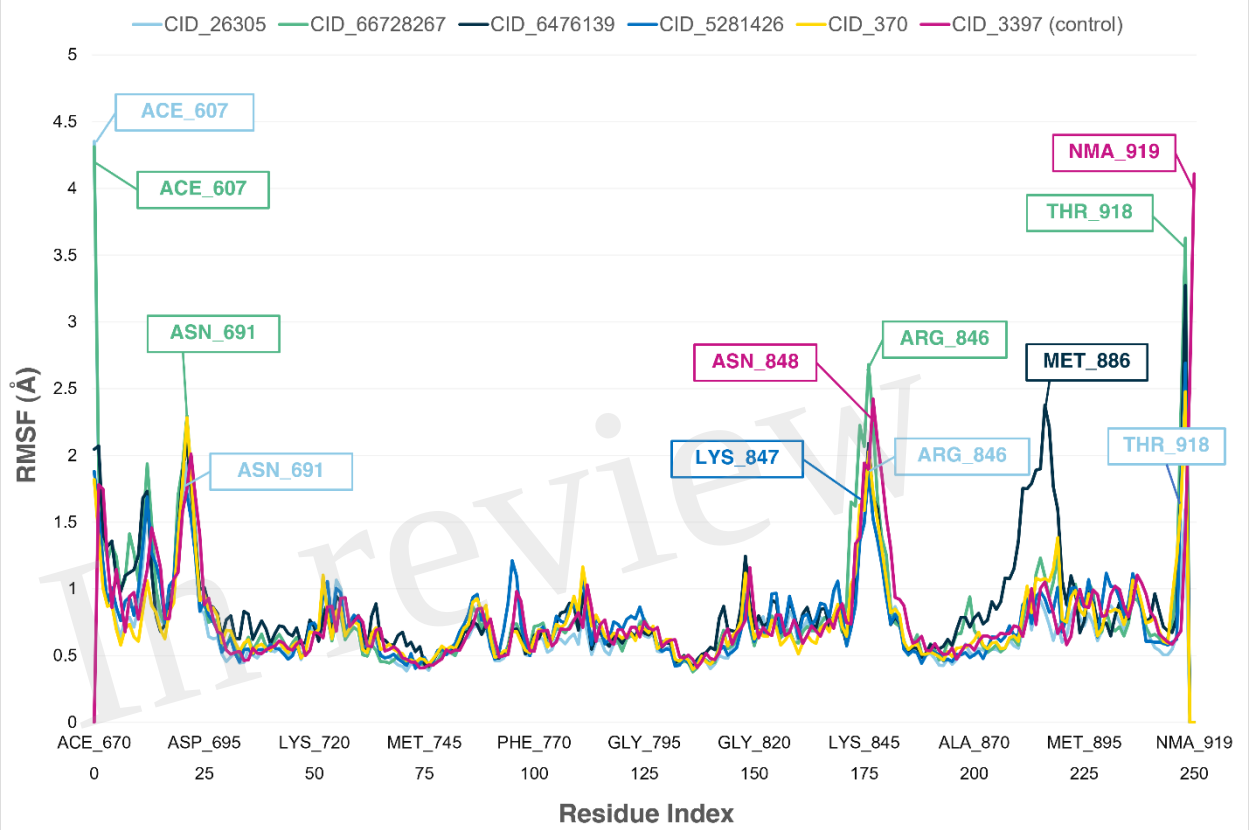

Figure 9: RMSF Analysis. CID: 26305 (sky blue), CID: 66728267 (green), CID: 6476139 (navy blue), CID:5281426 (blue), CID: 370 (yellow) and CID: 3397 (violet) to 100 ns simulation time.

Protein-ligand contacts analysis

Over the duration of the 100 ns simulation, protein interactions with the ligand may be observed. In the Figure 10, illustrates how these interactions can be divided into categories and summarized by type. The four types of protein-ligand interactions (or "contacts") appeared in the analysis including hydrogen bonds, hydrophobic interactions, ionic interactions, and water bridges. The subcategories of each interaction type were viewed using the SID module. Throughout the trajectory time frame, the stacked bar charts are normalized. However, at the residue position of Asn705, which connects to other molecules via hydrogen and water bridge connections, the stacked bar charts for phytochemical CID: 26305 (Nodakenetin) shows an interaction fraction value (IFV) of 0.78 Å, it suggests that the particular interaction has been sustained for more than 70% of the simulated time (Figure 10A). The IFV found a maximum of 1.2 Å at Phe764 position

that was produced by a hydrogen and water bridge bond as well as 0.98 Å at Asn705, which indicated that the CID: 66728267 (Isowigtheone hydrate) phytochemical was maintaining the contacts over 100% and 90%, respectively (Figure 10B). Additionally, the phytochemical CID: 5281426 (7-Hydroxycoumarin) has an IFV value of 0.97 Å at the position of Asn705, which was created by a hydrogen and water bridge bond that was kept at a level of above 90% contact (Figure 10D). The other two phytochemical are retaining low IFV and have produced many hydrogens, hydrophobic, water bridge bonds, and ionic bond interactions as well in comparison to the other three phytochemicals and the control drug. The IFV is low at Ala748 position in the case of CID: 6476139 (Methyl chlorogenate) phytochemicals, and the IFV value is optimized at Leu704 and Asn705 position as well (Figure 10C). The IFV is kept very low for the phytochemical CID: 370 (Gallic acid) at the positions of Gln711 and Met745, while the positions of Leu704 and Thr877 are similarly optimized for IFV value (Figure 10E). The phytochemical produced the least amount of hydrogen bonds with the desired androgen receptor; hence its stability should be compromised. Due to the sidechain of protein, it possesses more than one H-bond donor characteristic. The control drug CID: 3397 (Flutamide) was shown to form numerous contacts with the same residue within the same atom of the ligand (Figure 10F). However, the CID: 26305 (Nodakenetin) shows 76% of interactions in the Asn705 residue from 0 to 100 ns of the simulation time. Also, the highest interactions for phytochemicals CID: 6476139 (Methyl chlorogenate), CID: 5281426 (7-Hydroxycoumarin), and CID: 370 (Gallic acid) are 42% with Leu704, 92% with Asn705 and 70% with residue Thr877 respectively.

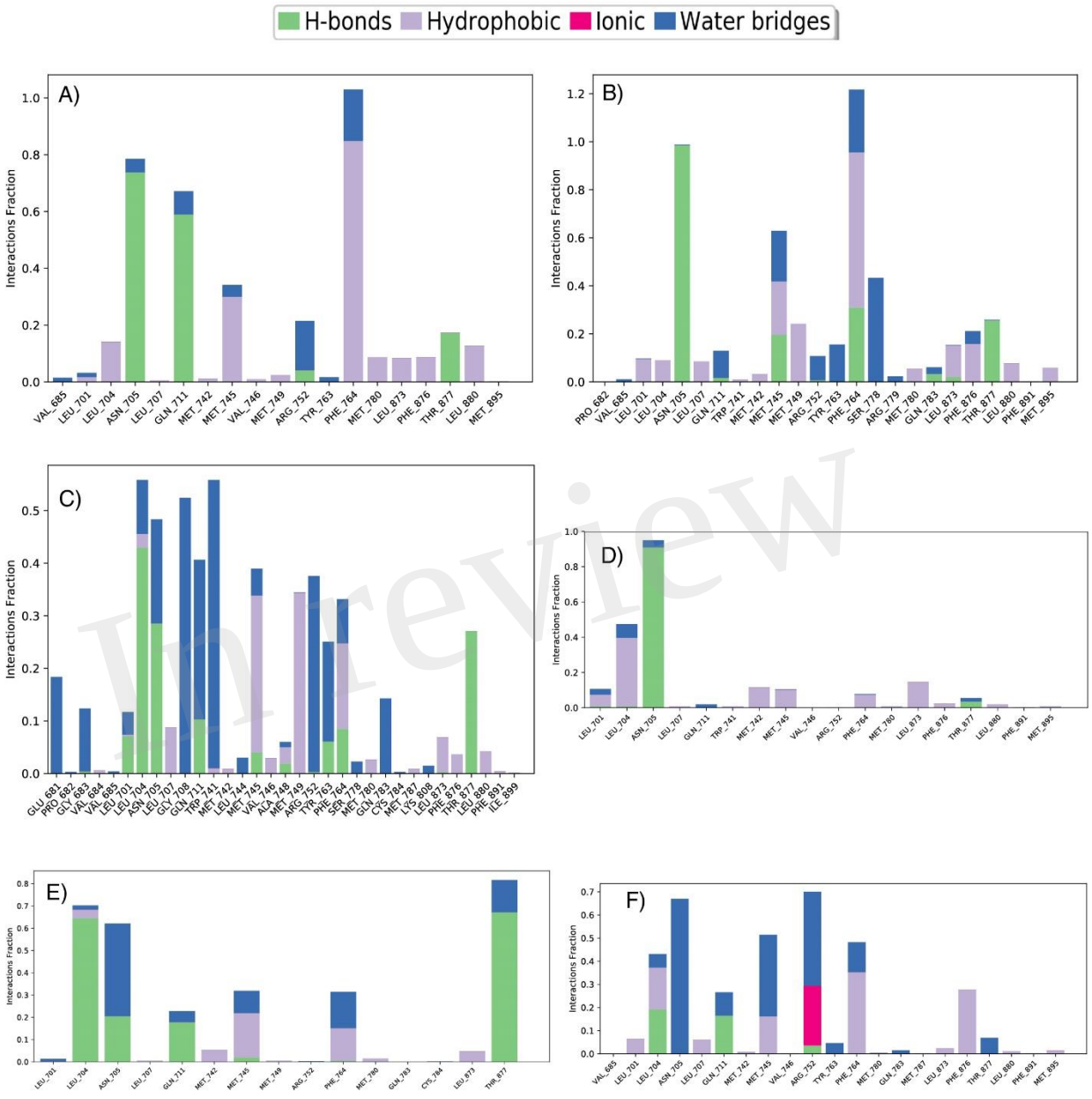

Figure 10: Protein-ligand contacts of (A) CID: 26305 (B) CID: 66728267 (C) CID: 6476139 (D) CID: 5281426 (E) CID: 370 (F) CID: 3397(control)

Ligand properties analysis

The Radius of Gyration (Rg), Molecular Surface Area (MolSA), Solvent Accessible Surface Area (SASA), Polar Surface Area (PSA of the phytochemicals have been analyzed for the receptor protein and protein-phytochemical complexes. Rg is used to determine the compactness of a protein. MolSA and SASA reveals the pharmacokinetic potentiality of ligands ((hydrophilic or

hydrophobic) as target specific towards protein macromolecules. The phytochemicals CID: 6476139 (Methyl chlorogenate) and CID: 66728267 (Isowigtheone hydrate) performed maximum fluctuations during the 100 ns simulation time (Figure 11A, B, C and D). All other phytochemicals are showing optimized stability but the phytochemical CID: 26305 (Nodakenetin) and CID: 5281426 (7-Hydroxycoumarin) both shows impressively stable with the receptor protein and well as drug molecules.

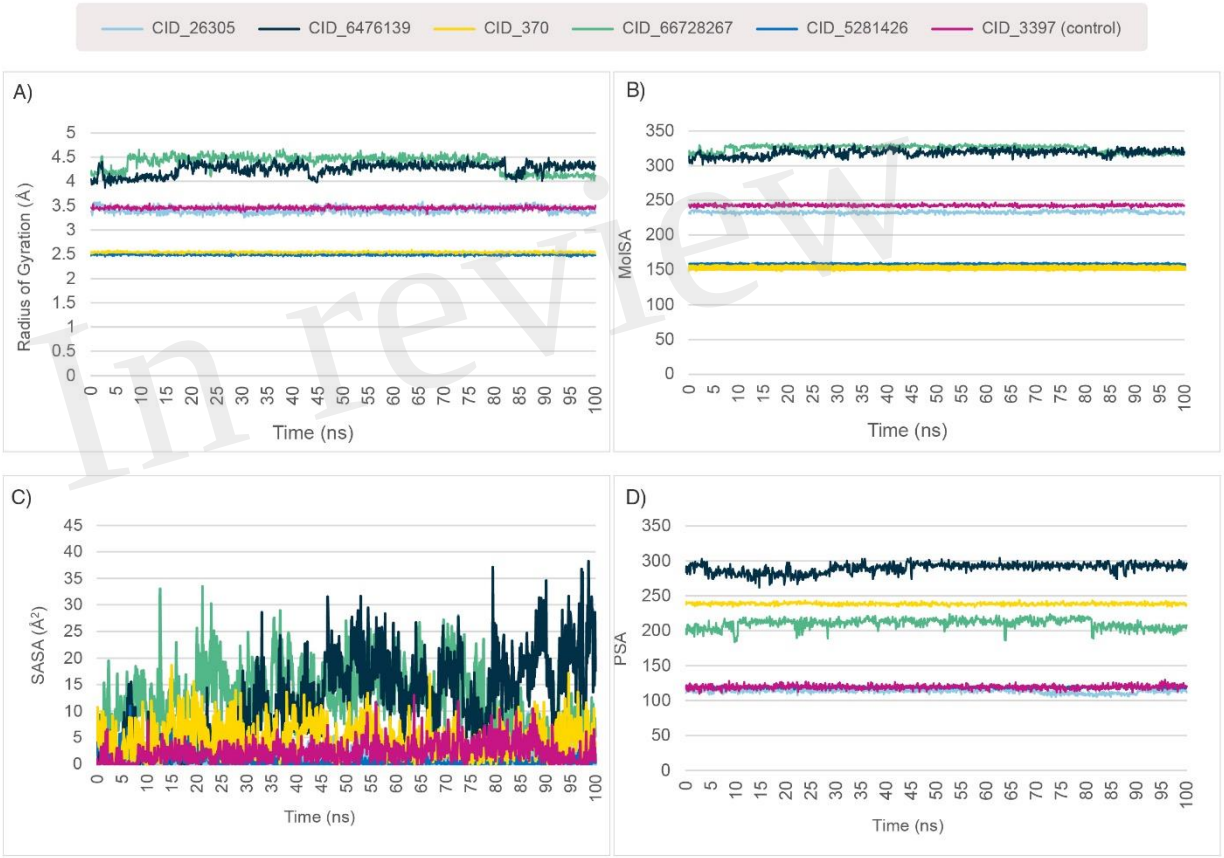

Figure 11: Ligand properties results of (A) radius of gyration (Rg) values of 5T8E androgen receptor, (B) denote the values of MolSA, (C) SASA, and (D) PSA

H-bond analysis

Hydrogen (H) bonds plays a key role in both the formation and stabilize of protein structures and stabilize a ligand with the targeted protein in a ligand-protein complex (Chikalov et al., 2011, Samad et al., 2022). In drug discovery study, H-bond influence the drug specificity and it accelerates its metabolism and adsorption, therefore, the stability of H-bond is crucial. In this study we analyzed the receptor protein-phytochemical complexes H-bond stability during the 100 ns

simulation runtime (Figure 12). Impressively, the results reveal that all the phytochemicals are maintaining H-bonding stability with the 5T8E androgen receptor at a optimize level during all the 100 ns runtime except the control drug CID: 3397 (Flutamide).

Figure 12: Hydrogen bond analysis result of (A) CID: 26305 (sky blue), (B) CID: 66728267 (green), (C) CID: 6476139 (navy blue), (D) CID:5281426 (blue), (E) CID: 370 (yellow) and (F) CID: 3397 (violet).

Discussion

In 2020, prostate cancer is the second most frequent cancer that occurs men death with its unique features of origin, mutations, gene expression pattern and overall health infections (Sung et al., 2021, De Silva and Alcorn, 2022). Prostate cancer is responsible for 1.4 million new cases of cancer in men and 375,000 deaths from the disease each year (De Silva and Alcorn, 2022). Androgen receptors (AR) are play an important role in the development of prostate cancer (Westaby et al., 2022, Dey et al., 2022). Targeting the AR and determining the mechanisms of resistance to these agents continue to be central goals of drug development efforts (Westaby et al., 2022). Whereas, *Ficus hispida* has highly cytotoxic effects on prostate cancer cell lines as well as androgen receptors (B. Pratumvinit and Kummalue, 2009). In this study, we demonstrated that *Ficus hispida* fruits extracted phytochemicals interact with the AR of prostate cancer and markedly down or up regulated the prostate gland expression. To the best of our knowledge, this exhaustive computational analysis has been carried out for the purpose of identifying potential drugs or drug-like candidates that can combat prostate cancer by targeting the AR 5T8E protein.

The term "computer-aided drug design," abbreviated as "CADD," refers to a set of computational methods that are utilized in the process of finding, developing, and analyzing drug molecules and active molecules that share similar biochemical properties (Sabe et al., 2021). CADD has sped up drug development by narrowing scientists' biological and synthetic research. The drug candidate identified through CADD approaches like ADMET, molecular docking and MD simulation that has the highest biological efficacy (Samad et al., 2022). ADMET has made it easier to screen retrieved phytochemicals from a database library whether molecular docking helps to predict the best binding mode of ligands with a specific receptor protein. MD simulations can be used to investigate ligand interactions at the atomic level in conjunction with the targeted receptor protein as a potential drug candidate for the treatment of a specific disease. (Yu and MacKerell).

We screened total 109 phytochemicals of *Ficus hispida* that extracted from different databases and literatures. This study focuses on the bioactive phytochemicals found in the fruits of *Ficus hispida* as it is popular as edible parts. Therefore only 13 bioactive phytochemicals were selected as potential ligand for the ADMET study. The ADME study provides the most comprehensive data set for analyzing how the drug is processed by the human body (Spracklin et al., 2020). During this study, only 10 ligands passed the ADMET test based on the considered parameters of molecular weight < 500, donor hydrogen < 5, acceptor hydrogen <10 and violation of rule of five

< 3. Indeed, molecular docking was performed with the selected ligands against the receptor protein 5T8E.

In this study, prostate cancer inhibitors were screened computationally. Binding affinities of selected ligands with the targeted prostate androgen receptor are evaluated based on docking scores and intermolecular hydrogen bonding interactions. Thus, top-scoring phytochemicals had three hydrogen bonds with amino acids in the binding pocket.

Molecular docking aims to understand and predict molecular recognition, both structurally and energetically (i.e., predicting binding affinity). In biology, hydrogen bond is essential for DNA structure and usually considered by adding an additional term to the binding energy of a complex (Stanzione et al., 2021, Islam et al., 2019). Based on the molecular docking results top seven ligands were selected considering their docking score (> -5) and number of hydrogen bonds. The activity of the selected ligands was checked using PASS online activity prediction. Pa > Pi parameter was set for selecting the ligands activity and activities only related to the anti-prostate cancer were considered. Finally, the five ligands were selected for molecular dynamic simulation based on their docking score and hydrogen bonds activities.

Molecular docking was carried out to evaluate the binding ability of the ligands to the 5T8E receptor protein. Docking score of CID: 26305 (Nodakenetin) was -9.946 and bound to the 5T8E receptor with two conventional hydrogen bonds in ASN 705 and ARG 752 residues. CID: 6476139 (Methyl chlorogenate) bound to the receptor with five conventional hydrogen bond and docking score was -8.685. CID: 66728267 (Isowigtheone hydrate) exhibited -9.199 and three conventional hydrogen bonds. Both CID: 370 (Gallic acid) and CID: 5281426 (7-Hydroxycoumarin) bound to the receptor with single conventional hydrogen bond and scored -7.457 and -7.653 respectively in molecular docking. All these five ligands found to have prostate cancer treatment activity in PASS online ligand activity prediction, though gallic acid and 7-hydroxycoumarin had lower Pa (<0.3) value. Another compound, 6-[(R)-2-Hydroxy-3-methyl-3-butenyl]-7-hydroxycoumarin scored better than other ligands in molecular docking but it was not considered for dynamic simulation for the lack of any anti-prostate cancer activity. The data of molecular docking indicates that the binding affinity of the selected ligands was higher, and the PASS online prediction confirms their possible potential against prostate cancer.

MD simulation explores protein–ligand binding stability (Hollingsworth and Dror, 2018). The MD simulation also provides intermolecular interaction information (Samad et al., 2022). Based on

RMSD, simulation equilibrium can be determined. On the contrary, fluctuations between 1–3 Å in a reference protein structure are acceptable, but larger values indicate large conformational change and instability. (Schreiner et al., 2012). The protein RMSD data depicts that all the selected ligands performed well, and their average fluctuation form the frame of protein backbone is 0.5 Å. The fluctuation from 1-3 Å is acceptable, and the range more than 3 Å is considered unstable. To check the stability of the ligands the ligand RMSD was calculated along with the control compound. In ligand RMSD CID: 26305 (Nodakenetin) and CID: 66728267 (Isowigtheone hydrate) showed more stability than other phytochemicals. Comparing to the control compound, CID: 6476139 (Methyl chlorogenate), CID: 5281426 (7-Hydroxycoumarin) and CID: 370 (Gallic acid) exceeded the acceptable fluctuation range (3 Å). Also, the ligand properties analysis specially the H-bond analysis revealed that the *Ficus hispida* fruits phytochemicals are able for the drug adsorption, metabolism, and specificity.

According to the similar androgen receptor (PDB ID: 5T8E) and anti-prostate cancer therapeutic studies, researchers suggested that the androgen receptor is a promising target for prostate cancer treatment (Ikwu et al., 2021, Ikwu et al., 2020, Bhati et al., 2019). Although no in vitro or in vivo studies have been performed for the treatment of prostate cancer with *Ficus hispida*, so this in silico study may serve as a starting point for future research. For instance, the *Ficus hispida* fruits selected phytochemicals CID: 26305 (Nodakenetin), CID: 66728267 (Isowigtheone hydrate), CID: 6476139 (Methyl chlorogenate), CID: 5281426 (7-Hydroxycoumarin) and CID: 370 (Gallic acid) have lead drug specificity. However, we had a limitation for the phytochemical activity prediction that the PASS online prediction score is not > 0.5. To find out whether these phytochemicals have the potential to fight prostate cancer, additional in vitro or in vivo studies are needed. Therefore, further research into it may someday lead to a significant therapeutic treatment for prostate cancer.

Author Contributions

M.H.R. designed the project. generating the data, analyzing the data, and wrote the manuscript. M.S.Z. and P.B. analyzing the data and wrote the manuscript. A.A.M.S., R.A.I.R and S.I. wrote the manuscript. B.G. and R.I. collected the data. M.J.U., M.A.H., M.A.R., W.K and B.K. supervised and reviewed the manuscript. All authors read and approved the final manuscript.

Funding

This research was supported by Korea Institute of Oriental Medicine (grant number KSN2021240), Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2020R1I1A2066868), the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2020R1A5A2019413), a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : HF20C0116), and a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : HF20C0038).

Acknowledgments

We express our gratitude to the ABEx Bio-Research Center for providing the necessary software, hardware, and bioinformatics lab support (https://research.abexbio.com/).

Conflict of Interest

The authors declare no conflict of interest.

References

B. PRATUMVINIT, T. S., P. WORAWATTANANON, N. OPARTKIATTIKUL, W. JIRATCHARIYAKUL & KUMMALUE, A. T. 2009. In vitro antineoplastic effect of Ficus hispida L. plant against breast cancer cell lines *Journal of Medicinal Plants Research,* 3**,** 255-261.

BAN, T., OHUE, M. & AKIYAMA, Y. Multiple grid arrangement improves ligand docking with unknown binding sites: Application to the inverse docking problem.

BANERJEE, P. P., BANERJEE, S., BROWN, T. R. & ZIRKIN, B. R. Androgen action in prostate function and disease.

BENET, L. Z., HOSEY, C. M., URSU, O. & OPREA, T. I. BDDCS, the Rule of 5 and drugability.

BHARADWAJ, S., DUBEY, A., YADAVA, U., MISHRA, S. K., KANG, S. G. & DWIVEDI, V. D. 2021. Exploration of natural compounds with anti-SARS-CoV-2 activity via inhibition of SARS-CoV-2 Mpro. *Briefings in Bioinformatics,* 22**,** 1361-1377.

BHATI, S., KAUSHIK, V. & SINGH, J. 2019. In Silico Identification of Piperazine Linked Thiohydantoin Derivatives as Novel Androgen Antagonist in Prostate Cancer Treatment. *International Journal of Peptide Research and Therapeutics,* 25**,** 845-860.

BIOVIA 2021. BIOVIA, Dassault Systèmes, BIOVIA Workbook, Release 2021; BIOVIA Pipeline Pilot, Release 2021, San Diego: Dassault Systèmes.

BRITO, M. A. D. 2011. Pharmacokinetic study with computational tools in the medicinal chemistry course. *Brazilian Journal of Pharmaceutical Sciences,* 47**,** 797-805.

CHENG, J.-X., ZHANG, B.-D., ZHU, W.-F., ZHANG, C.-F., QIN, Y.-M., ABE, M., AKIHISA, T., LIU, W.-Y., FENG, F. & ZHANG, J. 2020. Traditional uses, phytochemistry, and pharmacology of Ficus hispida L.f.: A review. *Journal of Ethnopharmacology,* 248**,** 112204.

CHIKALOV, I., YAO, P., MOSHKOV, M. & LATOMBE, J.-C. 2011. Learning probabilistic models of hydrogen bond stability from molecular dynamics simulation trajectories. *BMC Bioinformatics,* 12**,** S34.

COLLIER, T. A., PIGGOT, T. J. & ALLISON, J. R. 2020. Molecular Dynamics Simulation of Proteins. *In:* GERRARD, J. A. & DOMIGAN, L. J. (eds.) *Protein Nanotechnology: Protocols, Instrumentation, and Applications.* New York, NY: Springer US.

DE SILVA, F. & ALCORN, J. 2022. A Tale of Two Cancers: A Current Concise Overview of Breast and Prostate Cancer. *Cancers,* 14.

DE VIVO, M., MASETTI, M., BOTTEGONI, G. & CAVALLI, A. 2016. Role of Molecular Dynamics and Related Methods in Drug Discovery. *Journal of Medicinal Chemistry,* 59**,** 4035-4061.

DEY, A., SEN, S. & MAULIK, U. 2022. Study of transcription factor druggabilty for prostate cancer using structure information, gene regulatory networks and protein moonlighting. *Briefings in Bioinformatics,* 23**,** bbab465.

FILIMONOV D.A., L. 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**,** 444-457.

FRIESNER, R. A., MURPHY, R. B., REPASKY, M. P., FRYE, L. L., GREENWOOD, J. R., HALGREN, T. A., SANSCHAGRIN, P. C. & MAINZ, D. T. 2006. Extra Precision Glide: Docking and Scoring

Incorporating a Model of Hydrophobic Enclosure for Protein−Ligand Complexes. *Journal of Medicinal Chemistry,* 49**,** 6177-6196.

H.M. BERMAN, J. W., Z. FENG, G. GILLILAND, T.N. BHAT, H. WEISSIG, I.N. SHINDYALOV, P.E. BOURNE 2000. The Protein Data Bank *Nucleic Acids Research,* 28**,** 235-242.

HASHEMI, S., NOWZARI DALINI, A., JALALI, A., BANAEI-MOGHADDAM, A. M. & RAZAGHI-MOGHADAM, Z. Cancerouspdomains: comprehensive analysis of cancer type-specific recurrent somatic mutations in proteins and domains.

HOLLINGSWORTH, S. A. & DROR, R. O. 2018. Molecular Dynamics Simulation for All. *Neuron,* 99**,** 1129-1143.

HURTADO-BARROSO, S., TRIUS-SOLER, M., LAMUELA-RAVENTÓS, R. M. & ZAMORA-ROS, R. 2020. Vegetable and Fruit Consumption and Prognosis Among Cancer Survivors: A Systematic Review and Meta-Analysis of Cohort Studies. *Advances in Nutrition,* 11**,** 1569-1582.

IGUCHI, T., TAMADA, S., KATO, M., YASUDA, S., YAMASAKI, T. & NAKATANI, T. 2019. Enzalutamide versus flutamide for castration-resistant prostate cancer after combined androgen blockade therapy with bicalutamide: study protocol for a multicenter randomized phase II trial (the OCUU-CRPC study). *BMC cancer,* 19**,** 339-339.

IKWU, F. A., SHALLANGWA, G. A. & MAMZA, P. A. 2020. QSAR, QSTR, and molecular docking studies of the anti-proliferative activity of phenylpiperazine derivatives against DU145 prostate cancer cell lines. *Beni-Suef University Journal of Basic and Applied Sciences,* 9**,** 35.

IKWU, F. A., SHALLANGWA, G. A. & MAMZA, P. A. 2021. Ligand Based Design, ADMET and Molecular Docking Studies of Arylpiperazine Derivatives as Potent Anti-Proliferate Agents Against LNCAP Prostate Cancer Cell Lines. *Chemistry Africa,* 4**,** 71-84.

ISLAM, M. T., KHALIPHA, A. B. R., BAGCHI, R., MONDAL, M., SMRITY, S. Z., UDDIN, S. J., SHILPI, J. A. & ROUF, R. 2019. Anticancer activity of thymol: A literature-based review and docking study with Emphasis on its anticancer mechanisms. *IUBMB Life,* 71**,** 9-19.

JANSEN, M. C., BUENO-DE-MESQUITA HB FAU - FESKENS, E. J. M., FESKENS EJ FAU - STREPPEL, M. T., STREPPEL MT FAU - KOK, F. J., KOK FJ FAU - KROMHOUT, D. & KROMHOUT, D. Quantity and variety of fruit and vegetable consumption and cancer risk.

KENNEDY, T. 1997. Managing the drug discovery/development interface. *Drug Discovery Today,* 2**,** 436-444.

KIM S, C. J., CHENG T, ET AL. 2021. PubChem in 2021: new data content and improved web interfaces. *Nucleic Acids Res,* 49**,** D1388–D1395.

LITWIN, M. S. & TAN, H. J. The Diagnosis and Treatment of Prostate Cancer: A Review.

MORRIS, G. M. & LIM-WILBY, M. 2008. Molecular Docking. *In:* KUKOL, A. (ed.) *Molecular Modeling of Proteins.* Totowa, NJ: Humana Press.

POROIKOV, V. V., FILIMONOV DA FAU - IHLENFELDT, W.-D., IHLENFELDT WD FAU - GLORIOZOVA, T. A., GLORIOZOVA TA FAU - LAGUNIN, A. A., LAGUNIN AA FAU - BORODINA, Y. V., BORODINA YV FAU - STEPANCHIKOVA, A. V., STEPANCHIKOVA AV FAU - NICKLAUS, M. C. & NICKLAUS, M. C. PASS biological activity spectrum predictions in the enhanced open NCI database browser.

PRATHEESHKUMAR, P., SON, Y.-O., KORANGATH, P., MANU, K. A. & SIVEEN, K. S. 2015. Phytochemicals in Cancer Prevention and Therapy. *BioMed Research International,* 2015**,** 324021.

ROOS, K., WU, C., DAMM, W., REBOUL, M., STEVENSON, J. M., LU, C., DAHLGREN, M. K., MONDAL, S., CHEN, W., WANG, L., ABEL, R., FRIESNER, R. A. & HARDER, E. D. 2019. OPLS3e: Extending Force Field Coverage for Drug-Like Small Molecules. *Journal of Chemical Theory and Computation,* 15**,** 1863-1874.

SABE, V. T., NTOMBELA, T., JHAMBA, L. A., MAGUIRE, G. E. M., GOVENDER, T., NAICKER, T. & KRUGER, H. G. 2021. Current trends in computer aided drug design and a highlight of drugs discovered via computational techniques: A review. *European Journal of Medicinal Chemistry,* 224**,** 113705.

SAMAD, A., HUQ, M. A. & RAHMAN, M. S. 2022. Bioinformatics approaches identified dasatinib and bortezomib inhibit the activity of MCM7 protein as a potential treatment against human cancer. *Scientific Reports,* 12**,** 1539.

SASTRY, G. M., ADZHIGIREY M FAU - DAY, T., DAY T FAU - ANNABHIMOJU, R., ANNABHIMOJU R FAU - SHERMAN, W. & SHERMAN, W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments.

SAYEGH, N., SWAMI, U. & AGARWAL, N. 2021. Recent Advances in the Management of Metastatic Prostate Cancer. *JCO Oncology Practice,* 18**,** 45-55.

SCHREINER, W., KARCH, R., KNAPP, B. & ILIEVA, N. 2012. Relaxation Estimation of RMSD in Molecular Dynamics Immunosimulations. *Computational and Mathematical Methods in Medicine,* 2012**,** 173521.

SCHRÖDINGER-DESMOND 2021. Schrödinger Release 2021-2: Desmond Molecular Dynamics System, D. E. Shaw Research, New York, NY, 2021. Maestro-Desmond Interoperability Tools, Schrödinger, New York, NY, 2021.

SCHRÖDINGER-EPIK 2021. Schrödinger Release 2021-2: Epik, Schrödinger, LLC, New York, NY, 2021.

SCHRÖDINGER-LIGPREP 2021. Schrödinger Release 2021-2: LigPrep, Schrödinger, LLC, New York, NY, 2021.

SCHRÖDINGER-MAESTRO 2021. Schrödinger Release 2021-2: Maestro, S. (LLC, 2021).

SCHRÖDINGER-PRIME 2021. Schrödinger Release 2021-2: Prime, Schrödinger, LLC, New York, NY, 2021.

SPRACKLIN, D. K., CHEN, D., BERGMAN, A. J., CALLEGARI, E. & OBACH, R. S. 2020. Mini-Review: Comprehensive Drug Disposition Knowledge Generated in the Modern Human Radiolabeled ADME Study. *CPT: Pharmacometrics & Systems Pharmacology,* 9**,** 428-434.

STANZIONE, F., GIANGRECO, I. & COLE, J. C. 2021. Chapter Four - Use of molecular docking computational tools in drug discovery. *In:* WITTY, D. R. & COX, B. (eds.) *Progress in Medicinal Chemistry.* Elsevier.

SUNG, H., FERLAY, J., SIEGEL, R. L., LAVERSANNE, M., SOERJOMATARAM, I., JEMAL, A. & BRAY, F. 2021. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians,* 71**,** 209-249.

TESTA, U. A.-O., CASTELLI, G. & PELOSI, E. Cellular and Molecular Mechanisms Underlying Prostate Cancer Development: Therapeutic Implications. LID - 10.3390/medicines6030082 [doi] LID - 82.

TRAN, C., OUK, S., CLEGG, N. J., CHEN, Y., WATSON, P. A., ARORA, V., WONGVIPAT, J., SMITH-JONES, P. M., YOO, D., KWON, A., WASIELEWSKA, T., WELSBIE, D., CHEN, C. D., HIGANO, C. S., BEER, T. M., HUNG, D. T., SCHER, H. I., JUNG, M. E. & SAWYERS, C. L. 2009. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science,* 324**,** 787-90.

WESTABY, D., FENOR DE LA MAZA, M. D. L. D., PASCHALIS, A., JIMENEZ-VACAS, J. M., WELTI, J., DE BONO, J. & SHARP, A. 2022. A New Old Target: Androgen Receptor Signaling and Advanced Prostate Cancer. *Annual Review of Pharmacology and Toxicology,* 62**,** 131-153.

WHO. 2022. *Cancer* [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/cancer?fbclid=IwAR2lYmQDs4DYCaFdwiXZRfAcsuTu53u_471SflUTfMYhU4MkqjeWKQcDoqk [Accessed 7-22-2022 2022].

YU, W. & MACKERELL, A. D., JR. Computer-Aided Drug Design Methods.