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Molecular docking and dynamics simulation analysis of PDE5 inhibitor candidates for erectile dysfunction treatment

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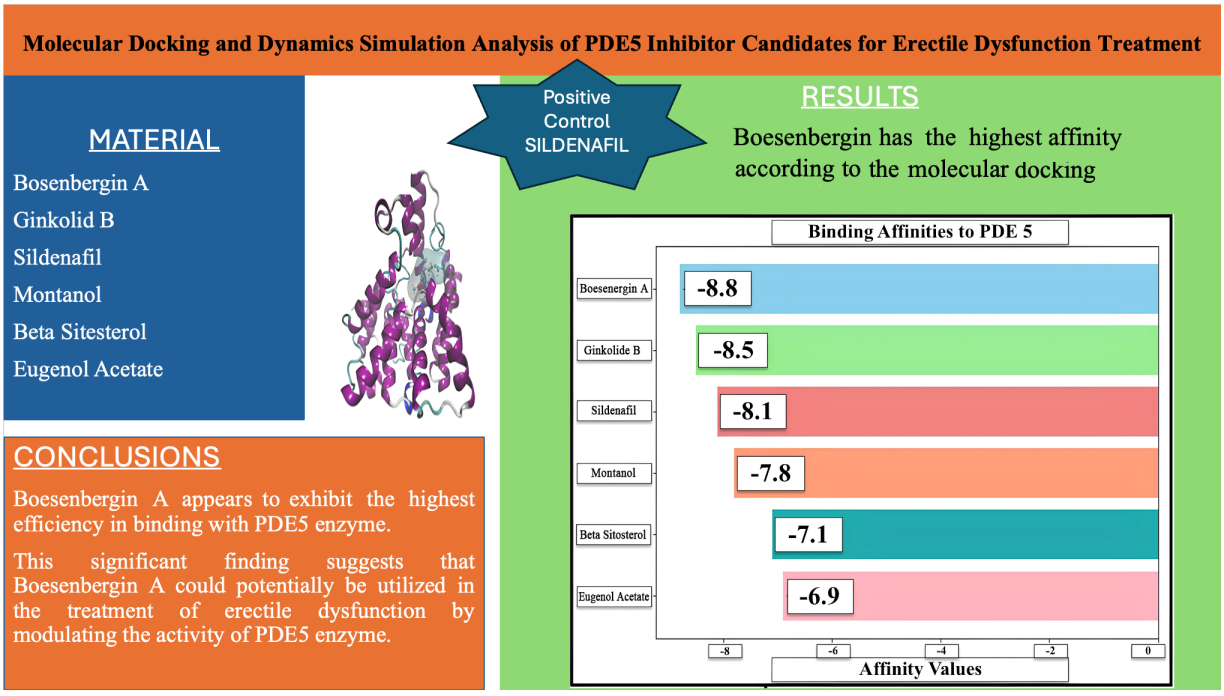
Abstract. **OBJECTIVE: Molecular docking studies were conducted to assess the binding affinities of five potential inhibitor candidates [PDB (Protein Data Bank) ID: 6L6E] against Phosphodiesterase 5 (PDE5), with Sildenafil used as the reference compound. The aim of this study is to reveal the potential inhibitory role of plant-derived compounds compared to Sildenafil, a PDE5 inhibitor.**

MATERIALS AND METHODS: Autodock Vina v. 1.2.5 software was used to dock the protein and each ligand individually. Molecular dynamics simulations assessed the binding affinity of two compounds to the Phosphodiesterase 5A1



Graphical Abstract. This study conducted a molecular docking and dynamics simulation analysis of PDE5 inhibitor candidates for the treatment of erectile dysfunction. The materials used in the research included Boesenbergin A, Ginkolid B, Sildenafil, Montanol, Beta-sitosterol, and Eugenol acetate. According to the molecular docking results, Boesenbergin A exhibited the highest binding affinity among the tested compounds. These findings indicate that Boesenbergin A has the highest efficiency in binding with the PDE5 enzyme. Therefore, this significant discovery suggests that Boesenbergin A could potentially be utilized in the treatment of erectile dysfunction by modulating the activity of the PDE5 enzyme.

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(PDE5 A1) enzyme and were carried out using GROMACS 2022.2

***RESULTS*: Boesenbergin A exhibited the highest affinity at -8.8 kcal/mol, followed by Ginkolide B at -8.5 kcal/mol, Sildenafil at -8.1 kcal/mol, Montanol at -7.8 kcal/mol, Beta-sitosterol at -7.1 kcal/mol, and Eugenol acetate at -6.9 kcal/mol, ranked in descending order. As a result of molecular docking studies, molecular dynamic simulations were performed for Boesenbergin A, which has the highest affinity, and Sildenafil, which is the standard molecule.**

***CONCLUSIONS*: Among the two ligands tested, Boesenbergin A exhibited superior binding affinity, surpassing even the standard molecule, Sildenafil. This suggests their potential for modulating enzyme activity and potential relevance in erectile dysfunction treatment.**

Key Words:

Molecular docking, Dynamics simulation, PDE5 inhibitor candidates, Erectile dysfunction treatment.

Introduction

Erectile dysfunction (ED) refers to the inability to attain penile erection, which has a substantial impact on the overall well-being and life quality of the affected individuals and their partners. ED becomes more common with age, affecting approximately 40% of men between 40 and 70 years old. This widespread issue impacts a significant number of men globally[1-3]. Among the most common reasons for erectile dysfunction are neurogenic and vascular factors, which become more noticeable and tend to increase with age, often occurring alongside other health issues such as hypertension, diabetes, atherosclerosis, high cholesterol, and metabolic syndrome. While vascular factors primarily affect erectile function at the local level, neurogenic factors can impact the nervous system at various levels, from local supply by the autonomic nervous system to the genital apparatus, to the spinal cord, higher brain centers, and even beyond[3-8]. An erection takes place after a series of reactions are set off by nitric oxide released from nerve cells. This process results in an increase of 3′,5′-cyclic guanosine monophosphate (cGMP), a versatile signaling molecule within cells. This, in turn, leads to the relaxation of the smooth muscle in blood vessels, ultimately enhancing blood flow to the penis. The rise in cGMP's impact is regulated by a diverse group of enzymes called phosphodiesterases (PDEs), with Phosphodiesterase 5A1 (PDE5A1) being the most prominent in penile erections[9,10].

Cyclic adenosine monophosphate (AMP) and cyclic GMP are degraded by phosphodiesterase (PDE) isoenzymes, a heterogeneous group of hydrolytic enzymes. 11 PDE isoenzyme families have been identified[11,12]. The ability of PDE5 inhibitors (PDE5i's) to induce penile erection has been considered a side effect when investigating treatments for hypertension and angina. ED is extremely common, with around 320 million people expected to be affected by 2025[13]. Oral PDE5 inhibitors are used worldwide as first-line treatment of ED with proven efficacy, tolerability, and dual satisfaction[14,15]. There are several PDE5i's on the market: FDA-approved Sildenafil (Pfizer, Brooklyn, NY, USA), Vardenafil (Bayer AG, Leverkusen, Germany), Tadalafil (ICOS Corporation, Bothell, WA, USA) and Avanafil (Mitsubishi Tanabe Pharma, Osaka, Kansai, Japan). Lodenafil (Cristália Produtos Químicos e Farmacêuticos, Itapira, Sao Paulo, Brazil), Udenafil (Dong-A, Gangseo-gu, Busan, South Korea) and Mirodenafil (SK Group, Jongno-gu, Seoul, South Korea) are other PDE5i's on the non-FDA market[16]. The widely known Sildenafil is the gold standard PDE5 inhibitor and has revolutionized the treatment of these conditions.

Even after a decade since the introduction of Sildenafil, ED remains underdiagnosed and undertreated in the context of internal medicine. This may be because patients perceive ED as a stress-related or age-related problem, which can make them hesitant to discuss it with their healthcare providers. While Sildenafil has proven to be highly effective, there is increasing interest in investigating alternative sources of PDE5i's, particularly plant-derived compounds[17,18]. Such findings could open new avenues for drug development and potentially lead to the discovery of novel PDE5i from natural sources. This research contributes to the ongoing investigation of natural products as potential therapeutic agents and highlights the importance of diversifying sources of PDE5i for better treatment options. The aim of this study is to reveal the potential inhibitory role of plant-derived compounds compared to Sildenafil, a PDE5 inhibitor. Sildenafil was used as the standard molecule in our study, which involved binding affinities and molecular dynamics simulations. Plant-derived molecules that are potential inhibitor candidates are Boesenbergin A, Ginkolide B, Montanol, Beta-sitosterol, and Eugenol acetate. As a result of *in silico* analyses, the most effective molecule identified as a potential inhibitor candidate on PDE5 is Boesenbergin A.

4421

Materials and Methods

Figure 1 shows the 2D structures of molecules that could be erectile dysfunction drug candidates. These structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov).

Molecular Docking

The 3D structures of the molecules were obtained from the relevant databases in pdb file format. The obtained PDE5 protein was first visualized using PyMOL (Schrödinger, Inc., New York, NY, USA) v. 2.5 software. At this stage, the presence of missing atoms in the protein, the presence of other ligands, and ions bound to it were checked, and the existing avanafil ligand was removed from the protein. The pdb file of the protein was transferred from Autodock Tools (The Scripps Research Institute, San Diego, CA, USA) v. 1.5.7 software to MGL Tools. Here, water molecules were removed from the protein, and polar bonds were added. The final version of the protein was saved in pdb file format. The saved PDB file was transferred to Swiss-Pdb Viewer v. 4.1 (Geneva, Switzerland) where the energy minimization of the protein was performed. The pdb file of the protein was then displayed in Autodock Tools v. 1.5.7 software, and a grid box was created for the docking process. Grid box X,Y,Z values; X center = -11.287, Y center = -24.304, Z center = -19.846, X size = 62, Y size = 54, Z size = 66. Autodock Tools V1.5.7 software was used to save the pdb files of the protein and ligands in pdbqt file format. Autodock Vina v. 2.5 software was used to dock the protein and each ligand individually. The affinity results of the ligands were saved in txt file format. As software output, the out.pdbqt file of each ligand and the out.pdbqt file of the protein was opened in PyMOL v. 2.5 software and the 1st conformation complex states were saved in pdb file format.

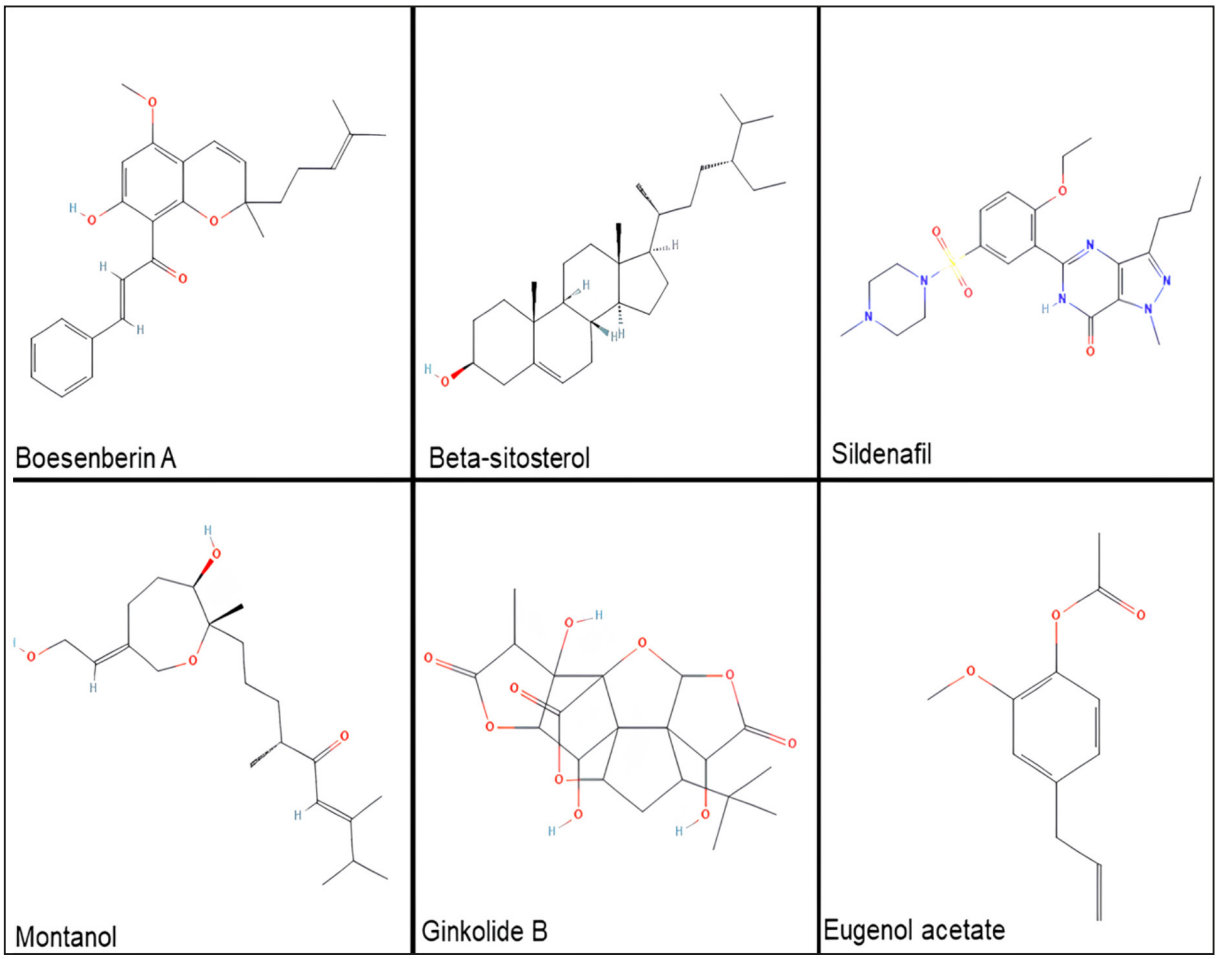

Figure 1. 2D structure of candidate molecules and the standard molecule Sildenafil.

Molecular Dynamic Simulation

Molecular dynamics (MD) simulation was carried out using GROMACS (University of Groningen, Groningen, Netherlands) 2022.2. The following steps were utilized.

Preparation of Enzyme

The 3D structures of ligand-protein complexes were exported in .pdb format using PyMOL. Their dynamic behavior was subsequently assessed through molecular dynamics (MD) simulations using the GROMACS package (version 2022.2)[19-21]. Protein topologies were generated with pdb2gmx employing the CHARMM27 force field[22], while ligand topologies were prepared using the SwissParam server[23] (Université de Lausanne, Lausanne, Switzerland).

Setting Up System for Simulation

Following the application of the force field, the complexes were incorporated into the system. They were solvated using the TIP3P water model[24] within a cubic box extending beyond 1 nm from the protein's edge, employing periodic boundary conditions. The system was neutralized by the addition of Na^+ ions, and energy minimization was performed for 50,000 steps using the steepest descent algorithm. This was succeeded by 100 ps of NVT (number of particles, volume, temperature) simulation at 300 K and 100 ps of NPT (number of particles, pressure, temperature) simulation to equilibrate the system. The Leapfrog algorithm was utilized in the constant-temperature, constant-pressure (NPT) ensemble to independently couple each component, including the protein, ligand, water molecules, and ions[25]. The Berendsen temperature and pressure coupling constants were set to 0.1 and 2, respectively, to maintain a stable environment at 300 K and 1 bar pressure[26]. Finally, a 100 ns MD simulation was conducted under isothermal and isobaric conditions at 300 K. Pressure coupling with a time constant of 1 ps was used to maintain a constant pressure of 1 bar, and the LINCS algorithm[27] was employed to constrain bond lengths. Van der Waals and Coulomb interactions were truncated at 1.2 nm, and the Particle Mesh Ewald (PME) algorithm[28] in GROMACS was used to minimize errors arising from truncation.

Visualization and Analysis of Simulation

The trajectory files were visualized using Visual Molecular Dynamics (VMD) (University of Illinois at Urbana-Champaign, IL, USA) version 1.9.2[29] and analyzed with the custom-developed tool HeroMDAnalysis[30,31] as well as Xmgrace version 5.1.25[32].

Results

Molecular Docking

Molecular docking studies were conducted on five molecules (PDB ID: 6L6E) identified as potential inhibitor candidates for the PDE5 enzyme. Sildenafil, a known PDE5i, was used as the standard for comparison. The affinity values, listed from highest to lowest, are as follows: Boesenbergin A (-8.8 kcal/mol), Ginkolide B (-8.5 kcal/mol), Sildenafil (-8.1 kcal/mol), Montanol (-7.8 kcal/mol), Beta-sitosterol (-7.1 kcal/mol), and Eugenol acetate (-6.9 kcal/mol), as illustrated in Figure 2.

Molecular Dynamic Simulations of Phosphodiesterase 5A1 (PDE5A1) Enzyme, in Complex with Boesenbergin A and Sildenafil

To evaluate the binding interactions of Boesenbergin A, which exhibited the highest affinity in the molecular docking results, and Sildenafil, used as a standard, with the PDE5 enzyme (PDB ID: 6L6E), we conducted 100 ns MD simulations for two models: Boesenbergin A-PDE5 and Sildenafil-PDE5 (standard), as shown in Figure 3. These simulations were analyzed using several statistical parameters, including Root-Mean-Square Deviation (RMSD), Root-Mean-Square Fluctuation (RMSF), and hydrogen bond formation, along with their respective percent occupancy throughout the simulation period.

RMSD analysis

Analyzing the RMSD offers crucial insights into the structural dynamics of both the protein and ligand throughout the simulation. Figure 4 illustrates a multiplot showing the RMSD trajectories for the protein and ligand over time across the two simulations. Significantly, both complexes achieved a stable conformation, as evidenced by protein RMSD values consistently below 0.3 nm.

RMSF analysis

RMSF examines localized changes within the protein structure. A multiplot in Figure 5 shows protein RMSF (measured in nanometres) as a function of residue number index.

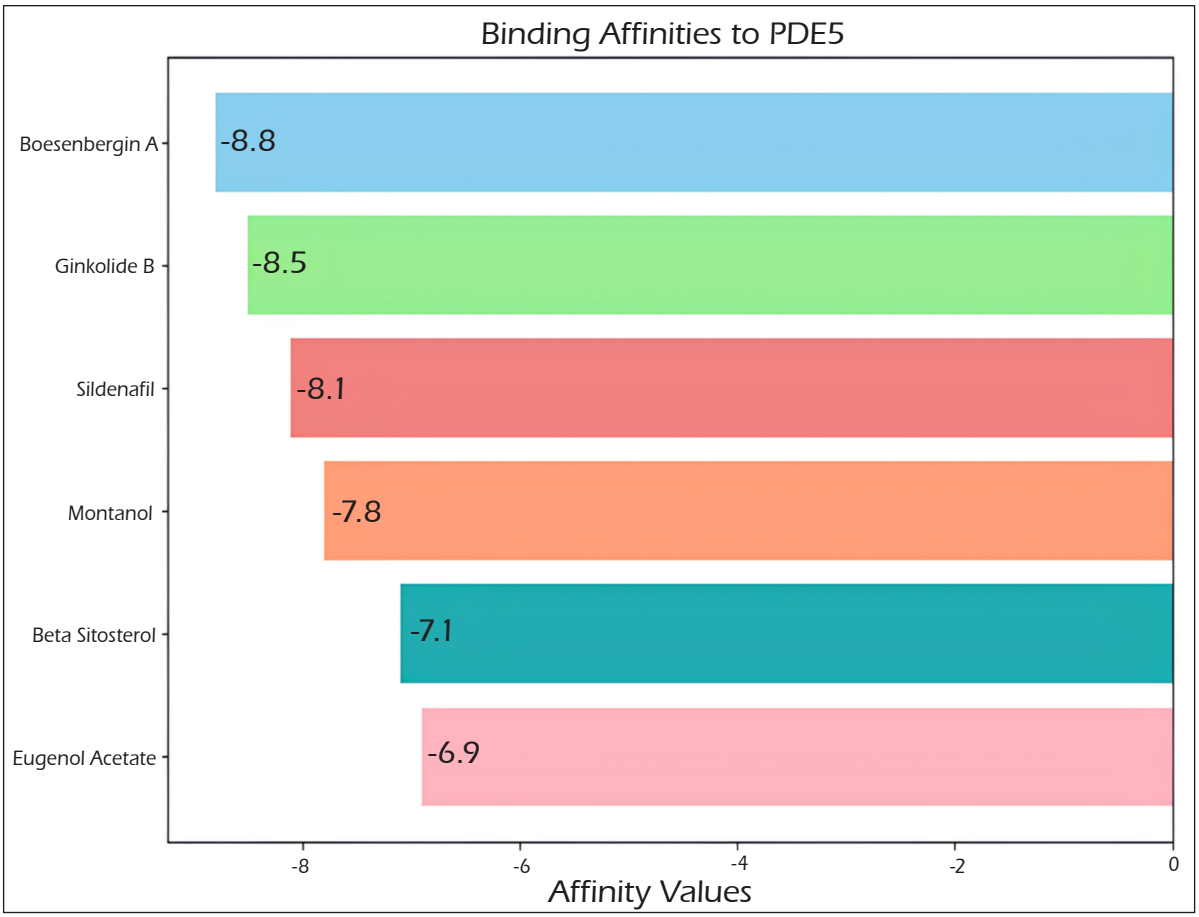

Figure 2. Graphical representation of the binding affinity values of 6 molecules obtained as a result of molecular docking with the PDE5 enzyme.

Hydrogen bond (h-bond) interaction

Molecular interactions, particularly hydrogen bond (h-bond) interactions, depend on both distance and angle parameters and are susceptible to disruption under dynamic conditions. In this study, we analyzed h-bond interactions for both complexes. Figure 6 presents a plot depicting the number of hydrogen bonds over time. Remarkably, the plot revealed that Boesenbergin A exhibited stronger and more consistent h-bond interactions throughout the simulation compared to the standard molecule, Sildenafil. To gain a deeper understanding of these interactions and evaluate their stability, we computed the percentage occupancies of specific residues involved in hydrogen bond interactions. Figure 7 presents a histogram illustrating the percentage occupancies of hydrogen bond contacts formed by different ligands. This analysis underscores Boesenbergin A's notable ability to establish robust interactions with ASP764 of the PDE5 enzyme, with an occupancy rate of 78.21%. In contrast, Sildenafil exhibits relatively weak hydrogen bond interactions with residues LYS812 and SER815, which were maintained for only 1.67% and 1.52% of the simulation period, respectively. These results indicate that, between the two ligands, Boesenbergin A demonstrates superior binding efficiency with the PDE5 enzyme.

ADMET Characteristics

Beta-sitosterol and Ginkolide B are characterized by good oral bioavailability, while Eugenol acetate has a negative effect with a high probability of eye corrosion. All molecules were found to have a high probability of hepatotoxicity and reproductive toxicity. Except for Ginkolide B, the level of nephrotoxicity is minimal among the molecules we performed docking studies. Eugenol acetate is the molecule with the highest probability of acute oral toxicity (Table I).

Table I. ADMET characteristics of molecules.

	HIA	HOB	EC	HEP	SS	RET	RPT	NET	AOT (c)	AOT (daily) Log [1/(mol/kg)]
Sildenafil	+	-	-	+	-	+	+	+	III	2.663
Beta-sitosterol	+	+	-	+	+	+	+	-	I	1.989
Boesenbergin A	+	-	-	+	-	-	+	-	III	2.107
Eugenol acetate	+	-	+	+	-	-	+	-	III	1.746
Ginkolide-B	+	+	-	+	-	+	+	+	III	2.888
Montanol	+	-	-	+	-	-	+	-	III	2.316

HIA: human intestinal absorption, HOB: human oral bioavailability, EC: eye corrosion, HEP: hepatotoxicity, SS: skin sensitization, RET: respiratory toxicity, RPT: reproductive toxicity, NET: nephrotoxicity, AOT: acute oral toxicity.

Discussion

PDE5 inhibitors are frequently used as first-line treatment for ED. There is a huge market and competition for PDE5i's. Since they are frequently used, they have become appealing to practitioners and those in the pharmaceutical industry. However, the search for new drugs continues at a rapid pace due to issues such as the fact that not every drug can be given to every patient, the common side effects and the cost of the drugs. The ultimate goal is to produce a drug with a minimal side effect profile, high bioavailability, few drug interactions, low cost and easy accessibility.

The herbal agents used in our study have already been used as traditional methods in the treatment of some diseases for many years. However, these agents should no longer be used as traditional methods but should be routinely used on a scientific basis with evidence-based medicine. However, it is also wrong to think that all of these molecules will be used as drugs. After the necessary *in vitro* and *in vivo* studies are performed for the most appropriate agents, clinical trials should be initiated.

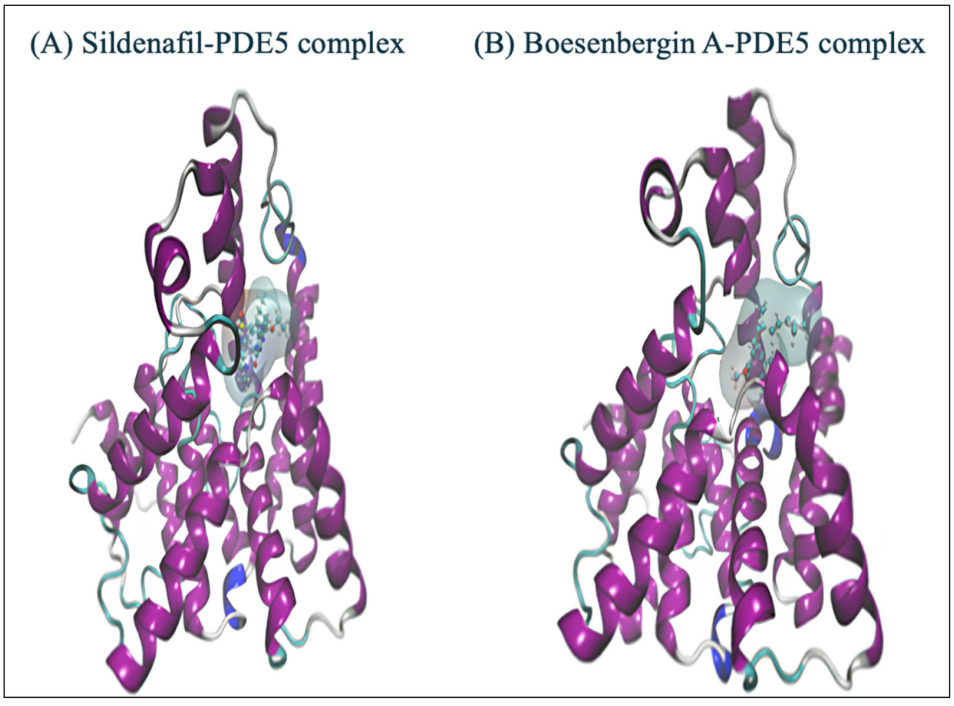

Figure 3. Graphical representation of protein-ligand complexes: (**A**) Sildenafil-PDE5 (standard) and (**B**) Boesenbergin A-PDE5 where the protein is shown in cartoon representation and the ligand is shown in Corey-Pauling-Koltun (CPK) representation with transparent surface.

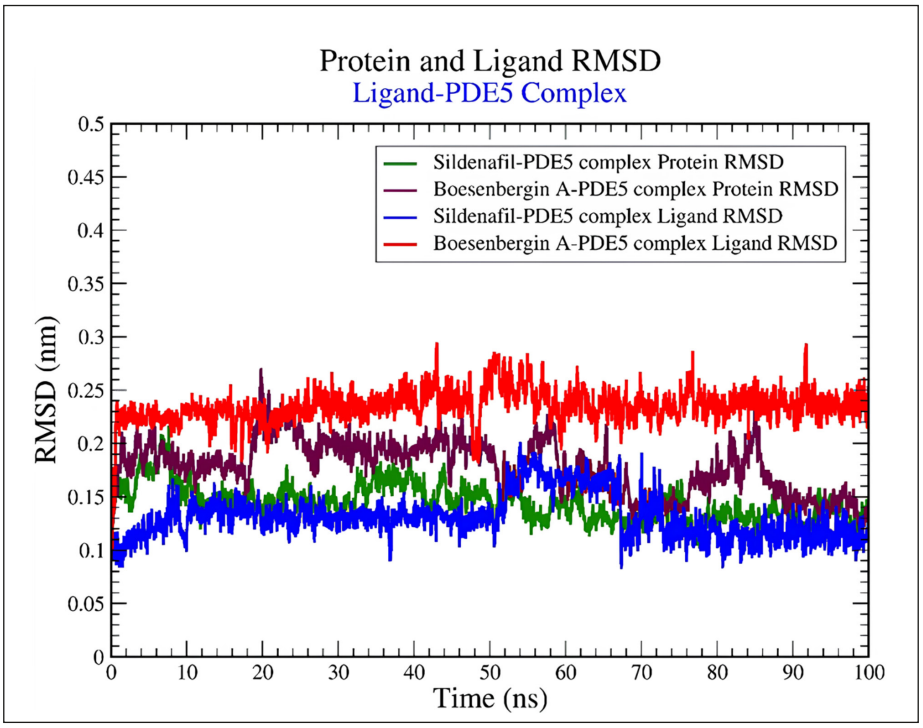

Figure 4. Graphical representation of the plots showing protein and ligand RMSD (nm) *vs.* time (100 ns) for Boesenbergin A-PDE5 and Sildenafil-PDE5 complex.

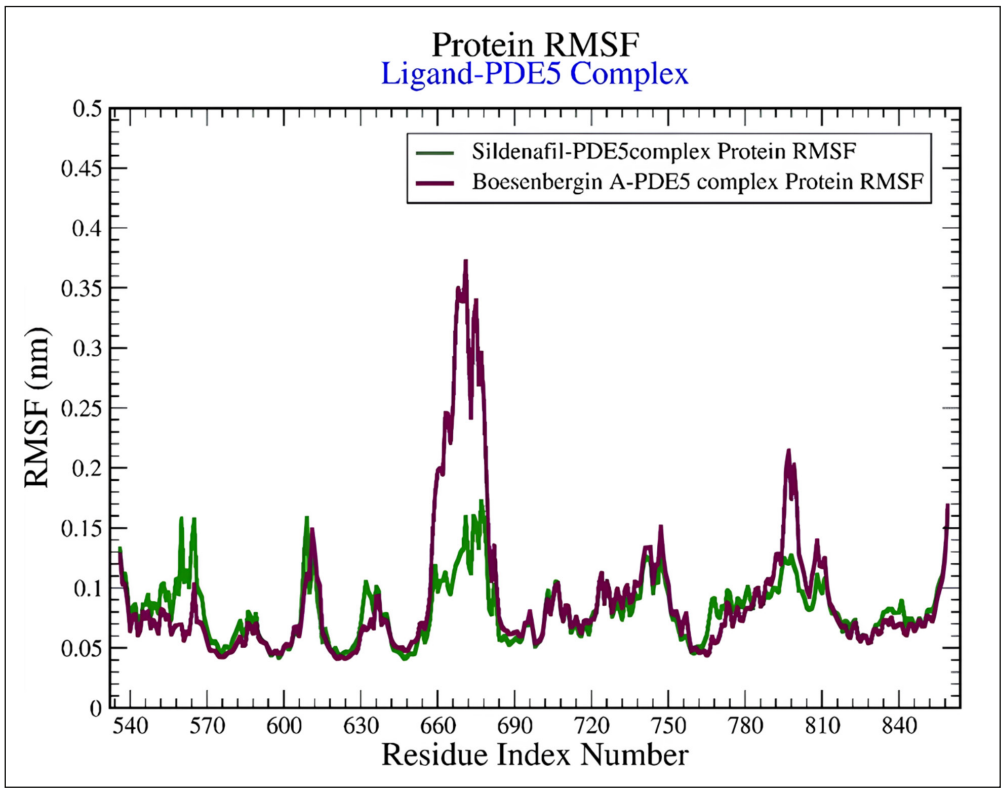

Figure 5. Graphical representation of the plots showing the protein RMSF (nm) *vs.* residue index number of protein for Boesenbergin A-PDE5 (green in color) and Sildenafil-PDE5 (maroon in color).

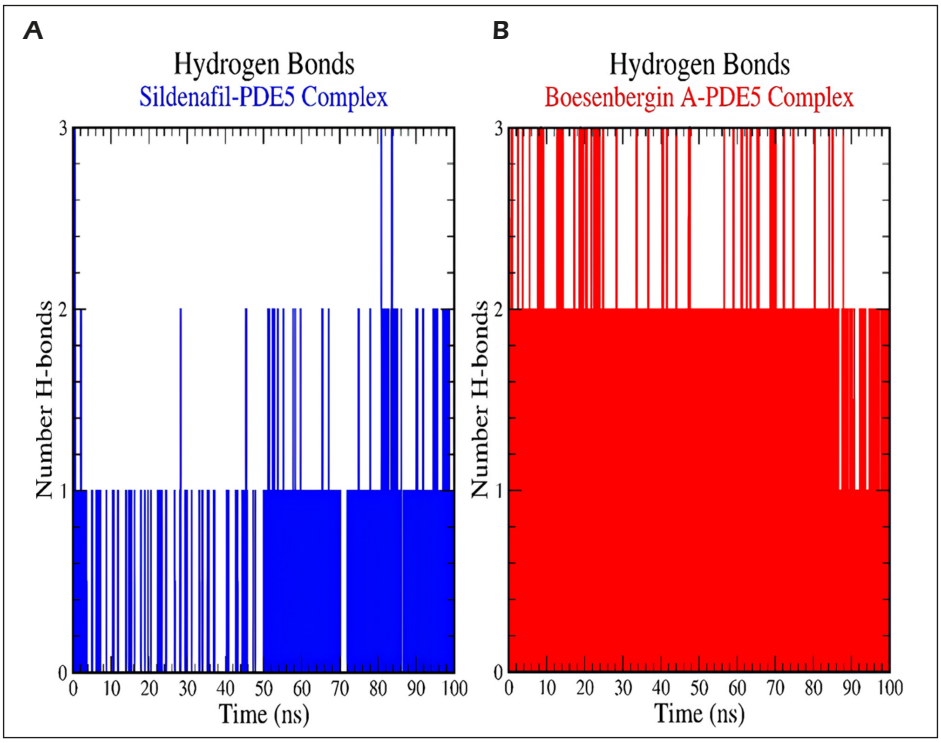

Figure 6. Pictorial representation of the number of h-bond contacts formed by ligands, (**A**) Boesenbergin A and (**B**) Sildenafil in complex with PDE5 enzyme (PDB ID: 6L6E).

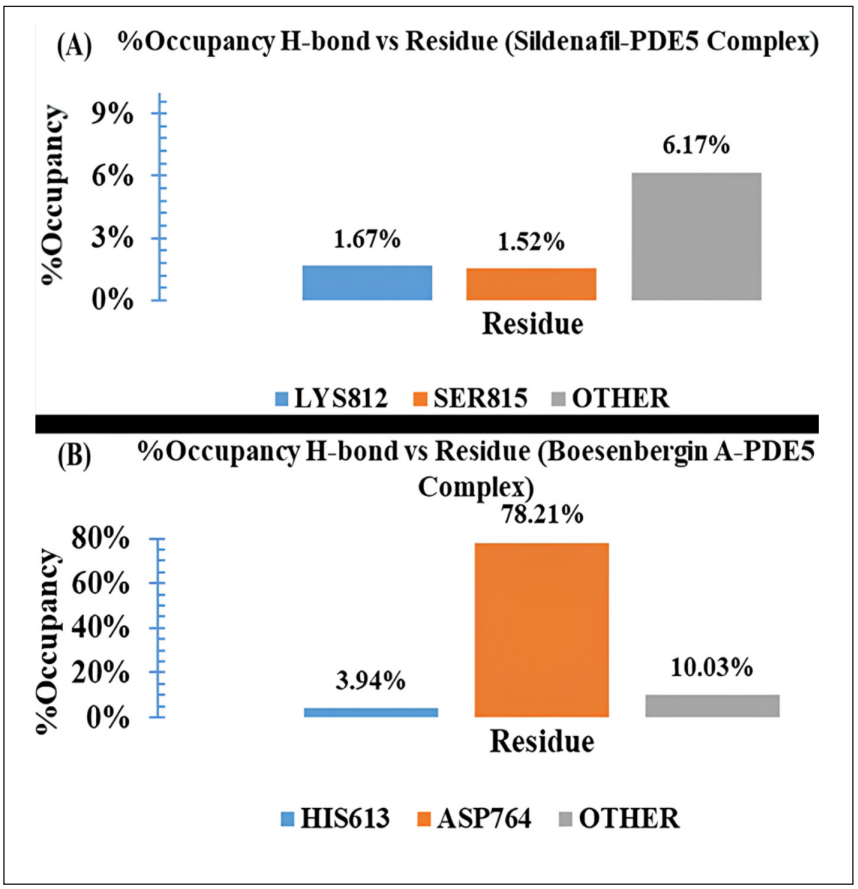

Figure 7. Histogram representation of % occupancies of the h-bond protein-ligand contacts of (**A**) Boesenbergin A and (**B**) Sildenafil in complex with PDE5 enzyme (PDB ID: 6L6E).

In this study, Boesenbergin A and Ginkolide B, which showed a higher affinity than the positive control Sildenafil, are promising. In addition, since the energies of the complexes were equal after the molecules bonded to the enzyme, we noticed that all the molecules we used in our study had strong inhibitory effects. The strong inhibitory effects indicate that each molecule can be considered as a drug candidate. However, Boesenbergin A and Ginkolide B, which show higher affinity, stood out. These molecules we used have been included in complexes whose aphrodisiac effects have been investigated as plant extracts in the literature[33] before, but they have not been investigated alone. Our study is, therefore, the first in the literature.

The RMSD values of the ligands provide insights into the stability of their binding with the protein. Figure 3 also displays the RMSD of the ligands over time for both simulations. Interestingly, both ligands exhibit RMSD values below 0.3 nm. However, it is notable that Sildenafil shows RMSD values of slightly lower magnitude compared to Boesenbergin A. Despite this, both ligands have demonstrated RMSD values within acceptable limits, indicating their ability to effectively bind with the PDE5 enzyme.

RMSF is a valuable tool for studying localized changes within the protein structures. It is important to emphasize that Figure 4 shows fluctuations lower than 0.4 nm for most protein residues, confirming the overall stability of the protein structure.

We know that the most important limiting factor in the daily use of medicines is their side effects. Drug choices are made by considering drugs with minimal side effects and contraindications. For this reason, ADMET properties such as absorption (A), distribution (D), metabolism (M), excretion (E) and toxicity (T) are as important as binding affinities and energies of complexes. Based on the ADMET properties revealed in our study, Table I shows that Ginkolide B has no nephrotoxicity; the bioavailability of the oral form of Boesenbergin A may be low and the respiratory toxicity of Montanol, Eugenol acetate and Boesenbergin A is minimal. These features are instructive and should be taken into consideration when conducting clinical trials.

In addition, the most recent study published by Dell'Atti et al[34] showed that PDE5 inhibitors could be used safely with combination therapies [in-plate injections, mechanical stretching, extracorporeal shock wave therapy ESWT (extracorporeal shock wave therapy)] not only in ED but also in Peyronie's disease. Urologists should be strongly encouraged to add PDE5 inhibitors to the treatment of this disease. This study is very valuable due to the long follow-up period and number of patients. It has been previously shown[35] that both nitric oxide (NO) and cGMP increase with PDE5 inhibition have an antifibrotic effect by inhibiting collagen synthesis. Strong inhibition is likely to make this effect more pronounced. It is possible to say that the Boesenbergin A molecule is particularly promising in this context. If these molecules are used as drugs in the future, their success in Peyronie's disease should be evaluated.

The results of this study will guide us for further research. Based on these findings, we are continuing the project by performing *in vitro* and *in vivo* studies of existing molecules.

Conclusions

To evaluate the binding affinity of Boesenbergin A and Sildenafil (standard) with PDE5 enzyme, we conducted an additional molecular dynamics simulation. The results strongly indicate that out of the two ligands, Boesenbergin A appears to exhibit the highest efficiency in binding with the PDE5 enzyme. This significant finding suggests that Boesenbergin A could potentially be utilized in the treatment of erectile dysfunction by modulating the activity of the PDE5 enzyme.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval

Since the study did not involve human or animal subjects and was performed in a computerized environment, ethical review and approval were waived for this study.

Informed Consent

Not applicable.

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AI Disclosure

No generative artificial intelligence was used to write the paper.

Authors' Contributions

Conceptualization: Tugrulhan Ozden, Fatıma Uzan.
Data curation: Fatıma Uzan, Mutlu Deger.
Formal analysis: Tugrulhan Ozden, Tunahan Ates.
Investigation: Tunahan Ates.
Methodology: Mutlu Deger.
Project administration: Ibrahim Atilla Arıdogan.
Resources: Ravi Rawat, Ismail Onder Yılmaz.
Software: Tugrulhan Ozden, Fatıma Uzan.
Supervision: Tunahan Ates, Ibrahim Atilla Arıdogan.
Validation: Ravi Rawat, Ismail Onder Yılmaz.
Visualization: Mutlu Deger.
Writing-original draft preparation: Tunahan Ates.
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Data Availability

All data generated or analyzed during this study are included in this published paper. The corresponding author may be contacted if necessary.

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