

Discovering a safer drug candidate from *Tribulus terrestris* for the management of erectile dysfunction through integrated in-silico studies

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Abstract

Conventional pharmacological therapies for erectile dysfunction (ED) primarily rely on PDE5 inhibitors such as Sildenafil, Tadalafil, Vardenafil, and Avanafil. While effective, these medications are reported to have side effects, especially among young men. Traditionally, plant-derived formulations with natural aphrodisiac potential have been used to treat ED, but they lack scientific validation. This study evaluates the efficacy of bioactive compounds from *Tribulus terrestris* in inhibiting the PDE5 enzyme located in the penile tissues, specifically smooth muscles of the corpus cavernosum. By selectively targeting PDE5, these compounds could effectively manage ED. Pharmacokinetic analysis of the phytochemicals from *T. terrestris* provides valuable insights into their suitability as drug candidates. The binding affinities of these phytochemicals with PDE5 were evaluated using *in-silico* approaches such as molecular docking and simulations. The top three phytochemicals from *T. terrestris*, Diosgenin, dehydro (-11.1 kcal/mol), Ruscogenin (-11.1 kcal/mol), and Hecogenin (-10.3 kcal/mol) showed higher negative binding affinities with the PDE5 enzyme, better than the control drug, Sildenafil (-8.8 kcal/mol). Hydrogen bonds and van der Waals interactions were the predominant forces influencing the interactions formed in the protein-ligand complexes. The ΔG_{bind} binding free energy for the top three phytochemicals, Diosgenin, dehydro, Ruscogenin and Hecogenin was found to be -19.99 ± 5.99 kcal/mol, -9.05 ± 5.16 kcal/mol, -14.11 ± 5.33 kcal/mol, respectively. Among the three, Diosgenin, dehydro, a saponin obtained from *T. terrestris*, is showing higher negative binding affinity and therefore is identified as a promising candidate for PDE5 inhibition, promoting prolonged erections by delaying the degradation of cGMP and preventing penile venous drainage. Pharmacokinetic analysis and toxicity assessments support their potency to be safer and effective alternatives to synthetic drugs. Furthermore, clinical validations are warranted to confirm their efficacy in humans.

1. Introduction

Erectile dysfunction (ED) refers to the persistent inability to achieve a satisfactory erection during sexual activity¹. ED was once mainly linked to aging, due to declining smooth muscle function. However, it now affects younger, healthy individuals, likely due to factors like stress, poor lifestyle, and chronic health conditions². The clinical manifestations of ED in young individuals are often correlated with various health conditions, including cardiovascular disease (CVD), benign prostatic hyperplasia (BPH), Type II Diabetes Mellitus (T2DM), and neurodegenerative disorders and psychological issues such as depression³⁻⁵. The cGMP-mediated nitric oxide (NO) pathway plays a pivotal role in penile erection. During sexual arousal, NO release stimulates guanylate cyclase to convert GTP into cGMP, leading to surging levels of cGMP in vascular smooth muscle cells present in the corpora cavernosa⁶. This results in smooth muscle relaxation, facilitating increased blood flow in the cavernosal arteries and subsequent filling of the sinusoids in the penis partially obstructing venous drainage and culminating in an adequate erection⁷. Terrett et al. discovered that Phosphodiesterase-5 (PDE5) is the primary cGMP-degrading enzyme located in the cytosolic fraction of the human corpus cavernosum⁸.

Phosphodiesterases (PDEs) catalyse the hydrolysis of the 3' cyclic phosphate bond on cyclic nucleotides, namely, cGMP and cAMP. PDEs are structured into three main domains: a highly conserved catalytic core, a regulatory N-terminus, and a carboxyl C-terminus. The N-terminal domains regulate and localize PDEs within the cell, involving ligand binding, PDE oligomerization, kinase recognition, and phosphorylation. The catalytic core, flanked by the N-terminal domains, has an active site formed by three helical subdomains: the N-terminal cyclic-fold region, the linker region, and the C-terminal helical bundle. This forms a deep hydrophobic pocket with four subsites: a metal-binding site (likely containing zinc or magnesium ions), a core pocket, a hydrophobic pocket, and a lid region. Metal ions stabilize the enzyme structure and activate hydroxide^{9,10}. Substrate specificity of PDEs depends on the rotation of a glutamine residue, GLN817, that forms hydrophobic bonds with cAMP or cGMP¹¹⁻¹³. These enzymes are categorized into 11 families based on structural similarities, which include sequence homology, protein domains, and enzymatic properties such as substrate specificity, kinetic properties, and responsiveness to endogenous regulators and inhibitors^{14,15}. Based on their amino acid sequences, some PDEs exhibit high specificity for the hydrolysis of cAMP: - PDE4, PDE7, PDE8; some are highly selective for cGMP: - PDE5, PDE6, PDE9; while other PDEs demonstrate mixed specificity, targeting both cAMP and cGMP: - PDE1, PDE2, PDE3, PDE10 and PDE11¹⁶. Among all the PDEs, PDE5's catalytic activity exerts inhibitory feedback on cGMP, which prevents cGMP breakdown, thereby increasing its intracellular concentration leading to penile venous drainage and thereby relieving erection^{15,17}. Sildenafil which is reportedly the first FDA-approved PDE5-inhibitor was discovered serendipitously as a treatment for ED since, originally it was developed to treat angina¹⁸. This innovative use of PDE5 inhibitors has since become a widely accepted treatment regimen for ED.

Pharmacologically, the most commonly used PDE5 inhibitors as first-line treatments of ED are FDA-approved Sildenafil, Tadalafil, Vardenafil, Avanafil, Apomorphine, synthetic Prostaglandin E1 (Alprostadil), and Phentolamine as well as non-FDA-approved medications like Lodenafil, Udenafil, Mirodenafil, and Papaverine^{6,19,20}. However, oral administration of PDE5 inhibitors is reported to have adverse effects, such as visual disturbances, headaches, facial flushing, rhinitis, indigestion, and hypotension in individuals concurrently taking organic nitrates^{6,21}. These effects primarily arise from the impairment of neural or endothelial NO production and the loss of trabecular smooth muscles. Since PDE5 is present in smooth muscle tissues, its inhibition can lead to non-specific accumulation of cGMP²². Most of the side effects result from the inhibition of other PDEs owing to their structural similarities, despite significant differences in their amino acid sequences outside the active site²³. Reports have indicated the cross-reactivity of PDE5 inhibitors with the catalytic site of PDE6, found in photoreceptor cells of the retina²⁴. Both Sildenafil and Vardenafil have been reported to cause visual disorders, including functional blindness, cyanopsia (blue-tinted vision), blurred vision, and increased light sensitivity²⁵. The use of these medications has also been associated with contraindications in high-risk cardiac patients, as they can target PDE3 resulting in elevated cAMP/cGMP levels in cardiac smooth muscle increasing cardiac contractility²⁶⁻²⁸.

Throughout history, various substances of plant origin have been utilized in folk medicines to enhance sexual function and physical performance in men ²⁹. Several herbs are renowned for their ability to energize and vitalize, addressing concerns related to ED and overall sexual health ^{30,31}. While many of these substances have not been extensively studied or identified pharmacologically, a few have gained recognition for their potential effect. *Tribulus terrestris*, an annual plant of the family Zygophyllaceae, has traditionally been used for generations to invigorate and enhance sexual function, physical performance, and vitality in men ³². There are three important species of *Tribulus*, namely *Tribulus cistoides*, *Tribulus terrestris*, and *Tribulus alatus*, found in India. Among them, *T. terrestris*, commonly known as Gokshur or Gokharu or puncture vine is a well-patronized medicinal herb and the fruits of *T. terrestris* have been utilized in the ayurvedic system of medicine for treating infertility, impotence, erectile dysfunction, and low libido ³³. It is reported to possess aphrodisiac properties attributed to phytochemicals like Protodioscin, which is 45% dry weight of *T. terrestris* extract for its purported ability to increase testosterone levels by stimulating the enzyme 5-alpha-reductase that converts testosterone into dihydrotestosterone ^{34,35}. Studies suggest *T. terrestris* stimulates spermatogenesis by increasing the production of Luteinizing Hormone (LH) from the pituitary gland, which enhances testosterone secretion and significantly improves both sperm quality and quantity ³⁶. In an *in vivo* study on male mice, the alcoholic and acetonitrilic extracts of *T. terrestris* showed a significant increase in free serum testosterone levels ³⁷. Additionally, the extracts were found to contain compounds that had minimal genotoxic effects on the germ cells of the mice ^{38,39}. Administration of *T. terrestris* in humans and animals has been shown to improve libido and spermatogenesis. In male rats, it increased sexual behavior and intracavernous pressure, and it also exhibited a pro-erectile effect on isolated rabbit corpus cavernosal tissues *in vitro* ^{40–42}. However, some studies have shown that *Tribulus* extract does not significantly impact serum testosterone levels or semen parameters in humans ⁴³. This contradiction could be due to other mechanisms, such as PDE5 inhibition, through which phytochemicals present in *T. terrestris* might enhance sexual performance. Phytochemicals in *T. terrestris* may have beneficial effects, but they may also be toxic because of their different chemical and physicochemical properties ⁴⁴. The plant's toxicity is attributed to steroidal sapogenins, which can form crystals in the bile ducts and renal tubules of sheep that consume its leaves ⁴⁵. Hence, our study has endeavoured to analyse selected phytochemicals in *T. terrestris* offering plant-based alternatives for ED treatment.

This study aims to identify and predict highly effective bioactive molecules from *T. terrestris* that have fewer known side effects compared to the currently available drugs for treating ED. Leveraging *in silico* methods, aids in the screening of potential drug candidates by using molecular docking against PDE5. The phytochemicals with favorable pharmacokinetics properties were identified through ADMET analysis. Further, Molecular Dynamics (MD) simulations assess the stability and binding interactions of selected phytochemicals with PDE5, contributing to the development of novel therapeutic strategies for ED management.

2. Materials and Method

2.1. Identification of phytochemicals

The study was initiated by accessing the Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT) database, a repository recognized for its extensive collection of phytochemical data related to Indian herbs ⁴⁶. Potential phytochemicals from various components of *Tribulus terrestris*, including the aerial parts, flowers, fruits, leaves, roots, seeds, stems and the whole plant has been meticulously identified from the IMPPAT database. Subsequently, a comprehensive ligand library was meticulously compiled, comprising phytochemicals sourced from both the IMPPAT database and relevant literature. To refine this library, lipid and lipid-like compounds, along with carbohydrates and carbohydrate conjugates were excluded from the initial pool of compounds resulting in the selection of a subset of phytochemicals for further investigation.

2.2. Ligand selection and preparation

The Structure Data File (SDF) of the list of 151 phytochemicals present in *T. terrestris*, retrieved from the IMPAAT database and 3D SDF structure of the control drug Sildenafil (CID- 135398744) was downloaded from the PubChem database ⁴⁷. As for ligand preparation, all 60 phytochemical compounds were converted from SDF to PDB file format using PyMOL ⁴⁸. The ligands were then saved in PDBQT format using the MGLTools v1.5.7 software for virtual screening ⁴⁹.

2.3. Target protein selection and preparation

The X-ray crystallographic structures of PDE5 bound to Sildenafil (PDB ID: 1UDT) with R and Rfree values of 0.193 and 0.251 respectively at a resolution of 2.30 Å were downloaded in PDB format from Protein Data Bank ^{50,51}. The 3D structure of PDE5 has missing residues from 665–677, which were added using SWISS-MODEL ⁵².

2.4. Molecular docking

Prior to docking, the target protein (PDE5) was prepared by eliminating water molecules, adding polar hydrogens, and then assigning Kollman charges. The ligands and protein were saved in PDBQT format using the MGLTools v1.5.7 software for virtual screening. Molecular docking of phytochemicals from *T. terrestris* against PDE5 was performed using AutoDock Vina v1.1.2 software ⁴⁹. Grid maps were generated with spacing was adjusted to 0.575 Å to facilitate ligand binding. The grid dimension was set to 40 × 40 × 40 Å in x, y, and z coordinates which could cover the catalytic domain of PDE5 with centre coordinates positioned at 1.440, 67.020, and 83.530 (Å), along the X, Y, and Z axes, respectively. The binding affinity of each ligand for the protein was calculated. The molecular docking output generates 10 configurations for each ligand. For each configuration, the binding affinity is calculated for the entire binding site with equilibrated energy distribution ⁵³. Binding affinity is derived from various factors, including hydrogen bonding, van der Waals forces, and electrostatic interactions. Higher negative binding affinity values indicate a stronger, more stable interaction, meaning the ligand fits well within the protein's active site, forming favorable interactions ⁵⁴. The complex with the highest binding affinity

(kcal/mol) was deemed the most suitable docking pose. Throughout the docking approach, exhaustiveness was fixed to 8 and was used to achieve more accurate and reliable results. The 3D interaction between ligands and protein was visualized and analyzed using PyMOL and a 2D protein-ligand interaction diagram was generated using the Ligand Interaction script in Maestro ^{55,56}.

2.5. ADMET Analysis

To comprehensively evaluate the pharmacological characteristics of Sildenafil and the phytochemicals present in *T. terrestris*, ADMET analysis has been conducted. The assessment of pharmacokinetic properties and drug-like characteristics of 60 phytochemicals was conducted by predicting ADME parameters using the SwissADME, a web-based tool ⁵⁷. SMILE notations for each phytochemical were retrieved from the PubChem database and used as input. This tool integrates computational prediction methods for a wide range of parameters, including intestinal absorption, aqueous solubility, blood-brain barrier penetration, plasma protein binding, cytochrome P-450 CYP2D6 inhibition, and Lipinski violations. The bioavailability was assessed using various parameters: lipophilicity (XLOGP3), molecular size (molecular weight, MW), polarity (topological polar surface area, TPSA), solubility (logS), saturation (fraction of Csp3), and flexibility (number of rotatable bonds). The acceptable ranges for these values were as follows: XLOGP3 between 0.7 and 5.0, MW ranging from 150 to 500 g/mol, TPSA between 20 and 130 Å², logS of 6 or less, a Csp3 fraction between 0.25 and 1, and no more than 10 rotatable bonds. The optimal range of values is as follows: XLOGP3 (– 0.7 to + 5.0), molecular weight (MW) (150–500 g/mol), topological polar surface area (TPSA) (20–130 Å²), solubility (logS) (≤ 6), fraction of Csp3 (0.25–1), and the number of rotatable bonds (≤ 10).

To validate our initial ADMET analysis, the pharmacokinetic and ADMET properties of the top three compounds (Ruscogenin; Diosgenin, dehydro; Hecogenin) obtained from *T. terrestris*, as well as the control substance Sildenafil, were analyzed using the pkCSM tool ⁵⁸. Lipinski's rule of five was applied to predict drug-likeness. Parameters such as Caco2 permeability, skin permeability, and intestinal absorption were assessed to evaluate absorption nature. Interaction with P-glycoprotein (P-gp) was analyzed, and P-glycoprotein inhibition potential was evaluated. Additionally, the volume of distribution (VD), blood-brain barrier (BBB) permeability, and inhibitory capabilities on different isoforms of Cytochrome P450 (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) were investigated. Toxicity studies, including AMES study, maximum tolerated dose, skin sensitivity, cardiotoxicity, hepatotoxicity, and hERG I and II inhibition, were conducted using ProTox-II ⁵⁹.

2.6. Molecular Dynamic Simulation

MD simulation studies were conducted to assess the binding stability, conformation, and interaction modes between selected ligands and the target protein PDE5. Ligands with the highest negative binding affinity to PDE5 were chosen for MD simulation studies. MD simulations of the receptor-ligand complexes were carried out for 200 ns using GROMACS (Groningen Machine for Chemical Simulations, version 2022.3) ⁶⁰. The topology of the selected ligands was obtained from the CGenFF server ^{61–63}, while the topology of the protein was prepared using CHARMM36 all-atom force field ver. July 2022 ^{64,65}.

The simulation system was placed in a dodecahedron periodic box with a distance of 1.0 nm from the box edge. The protein was solvated with a TIP3P water model, sodium and chloride counter ions were added to maintain the salt concentration in the system at 0.15 M. During the MD simulation, the initial vacuum was minimized using the steepest descent approach for 5000 steps to reduce energy. Canonical (NVT) and Isobaric (NPT) constraints were then applied for 1 ns, using two coupling groups (protein-ligand and water-ions) to equilibrate the system. Subsequently, each complex was simulated for 200 ns in the NPT ensemble (Isothermal-Isobaric, with a constant number of particles, pressure, and temperature).

Analysis of the MD trajectories was performed using the built-in utilities of GROMACS, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and solvent accessible surface area (SASA). Trajectory analysis results were visualized using the Matplotlib data visualization package. H-bond analysis was performed using gmx hbond to identify the residues that form hydrogen bonds with the ligand, the data was then conceptualized using the Matplotlib data visualization package through an event plot.

2.7. Molecular Mechanics Poisson–Boltzmann Surface Area (MMPBSA) Calculation

The MMPBSA approach was employed to calculate end-state free energy using MD trajectories, facilitated by gmx_MMPBSA⁶⁶. A per-residue decomposition analysis was conducted to elucidate the energy contribution of individual amino acids involved in ligand binding. Energy contributions from the protein, ligand, and complex were computed for each of the 1000 frames. The MMPBSA_ana module was used to visualize the results obtained from the gmx_MMPBSA run.

2.8. Principal component analysis (PCA)

PCA is utilized to explore the conformational differences and perturbations of the protein-ligand complex by examining equivalent residues. The resulting projection of principal components onto a subspace illustrates the percentage of total mean square displacement (variance) of atom positional fluctuations, characterized by their corresponding eigenvalues. The top three principal components are depicted in a conformer plot, while the proportion of variation versus the rank of the eigenvalue is presented in a scree plot. For PCA analysis, the R package BIO3D structural bioinformatics was used⁶⁷.

3. Results

3.1. Phytochemical's identification

A comprehensive ligand library created for the total 151 phytochemicals sourced from both the IMPPAT database and a thorough review of the existing literature is provided in Supplementary Table 1. The filtered 60 compounds are listed in Supplementary Table 2.

3.2. Molecular Docking studies

Molecular docking was conducted for all 60 compounds to assess their binding affinity towards PDE5. Among these compounds, Diosgenin, dehydro, and Ruscogenin have the highest docking score of -11.1 kcal/mol. The binding affinity of the top three phytochemicals and the control drug, Sildenafil, along with their respective interacting residues, is given in Table 1. The protein-ligand interactions depicted in Fig. 3 illustrate the binding and interactions formed by the top three phytochemicals and Sildenafil (control). The binding affinity of Sildenafil is -8.8 kcal/mol and formed polar interactions (HID613, HID617, SER766, GLN775, GLN817, ASN662, ASN661, HID657, THR723) and charge-charge interactions (ASP724, ASP654, GLU785, ASP764, GLU682) and 10 hydrophobic bonds (TYR612, LEU725, VAL782, PHE786, LEU765, ALA767, ILE768, PHE820, LEU804, MET816, TYR664, MET681) as shown in Fig. 3 (A). The results indicate that the phytochemicals Diosgenin, dehydro and Ruscogenin and their binding affinities were - 11.1 kcal/mol. Ruscogenin formed one hydrogen bond (GLN817), 10 hydrophobic interactions, PHE820, ILE768, ALA767, LEU765, MET681, VAL660, LEU725, TYR664, TYR612, VAL782, PHE786, ALA779, ILE778, TRP853 and polar interactions with six amino acid residues (GLN817, GLN775, SER766, HIS617, HIS653, HIS685, HIS657, ASN661, ASN662, THR723, HIS613) and charge-charge interactions with ASP764, ASP654, GLU682, ASP724 with PDE5 as shown in Fig. 3 (B). Diosgenin, dehydro outlines a highly hydrophobic cavity consisting of 10 proximate amino acid residues forming hydrophobic interactions, LEU725, PHE820, LEU765, ALA767, ILE768, ILE778, ALA779, TYR612, VAL782, PHE786, TYR664 and polar interactions with six amino acid residues (THR723, HID617, GLN817, GLN775, HID613, ASN662, ASN661) and charge-charge interactions with ASP724, ASP654, ASP764 with the target protein PDE5, as shown in Fig. 3 (C). Hecogenin binds the target protein PDE5 with a binding affinity of - 10.3 kcal/mol. Hecogenin made contact with 10 hydrophobic interactions (MET681, LEU725, LEU765, TYR612, PHE820, ALA767, ILE768, ILE778, ALA779, VAL782, PHE786, TYR664) and six polar interactions (THR723, HID657, HID617, HID613, GLN817, GLN775, ASN662, ASN661) and charge-charge interactions with GLU682, ASP724, ASP654, ASP764 as shown in Fig. 3 (D). The involvement of GLN817 in hydrogen bonding, along with PHE820, PHE786, and TYR664 forming well-directed bonds, contributes to the ligand's stability. The top three phytochemicals consistently interact with PHE820, LEU765, TYR612, ILE768, ALA779, GLN817, HID613, ASP724, all located within 5 Å of the ligand, enabling non-covalent interactions like van der Waals forces and π - π stacking. Notably, PHE820 is known for its role in stabilizing tetracyclic rings through π - π interactions, which is crucial for maintaining the inhibitory effect. Other residues, including PHE786, VAL782, LEU765, HIS685, and HIS613, also show a high probability of binding, further strengthening the interaction network. Hydrophobic residues like VAL782 and LEU765 help create a favorable environment for non-polar interactions, while aromatic residues such as PHE786 contribute to additional stabilizing π - π interactions.

Table 1

The binding affinities and interacting amino acid residues of the top three phytochemicals of *Tribulus terrestris* against PDE5

Compound name	Binding affinity (kcal/mol)	H-bond forming Residues	Residues binding through other interactions
Sildenafil (control)	-8.8	-	TYR612, LEU725, VAL782, MET681, TYR664, MET816, LEU804, PHE820, ILE768, ALA767, LEU765, PHE786 (Hydrophobic) HID613, THR723, HID657, ASN661, ASN662, GLN817, GLN775, SER766, HID617 (Polar) ASP724, ASP654, GLU682, ASP764, GLU785 (charged -ve)
Diosgenin,dehydro	-11.1	-	LEU725, PHE820, LEU765, ALA767, ILE768, ILE778, ALA779, TYR612, VAL782, PHE786, TYR664 (hydrophobic) THR723, HIS617, GLN817, GLN775, HID613, ASN662, ASN661 (polar) ASP724, ASP654, ASP764 (charged -ve)
Ruscogenin	-11.1	GLN281	PHE820, ILE768, ALA767, LEU765, MET681, VAL660, TYR664, LEU725, TYR612, VAL782, PHE786, ALA779, ILE778, TRP853 (Hydrophobic) GLN817, GLN775, SER766, HIS617, HIS653, HIS685, HIS657, ASN661, ASN662, THR723, HIS613 (Polar) ASP764, ASP654, GLU682, ASP724 (charged -ve)
Hecogenin	-10.3	-	MET681, LEU725, LEU765, TYR612, PHE820, ALA767, ILE768, ILE778, ALA779, VAL782, PHE786, TYR664 (Hydrophobic) ASN661, ASN662, GLN817, GLN775, HIS613, HID617, HID657, THR723 (Polar) GLU682, ASP724, ASP654, ASP764 (charged -ve)
Docking analysis in Autodock Vina assessed binding energy (kcal/mol). The top three phytochemicals that demonstrated the highest docking scores and favorable interactions compared to Sildenafil (control) are listed here.			

3.2 ADMET analysis

Assessment of pharmacokinetic properties and drug-likeness was carried out for the filtered 60 phytochemicals by predicting ADMET parameters. Diosgenin,dehydro, Ruscogenin, and Hecogenin emerged as the top three hits from ADMET analysis. The drug-likeness of the top three phytochemicals

with comparison to the Sildenafil (control) were predicted and are presented in Table 2 and the properties of each ligand and the control were visualized using bar plots in Fig. 2 (A).

Absorption nature of the selected ligands was studied using parameters such as Caco2 permeability, skin permeability, and intestinal absorption. None of the selected ligands showed the predicted value of > 0.90 except the Sildenafil (control) indicating low Caco2 permeability. The results indicated that all the selected ligands showed greater intestinal absorption (i.e.) $> 30\%$. Diosgenin, dehydro showed the highest intestinal absorption rate with 98.678% . When comparing Ruscogenin to Sildenafil (control) in terms of their interaction with P-glycoprotein (P-gp), it appears that Ruscogenin acts as a substrate for P-gp, indicating that it is recognized and transported by this protein across cell membranes. Results from the current study illustrate that all the selected ligands and the control have Log Kp value < -2.5 indicating favorable skin permeability.

Current studies suggest that all the selected ligands and the Sildenafil (control) are Class 1 P-glycoprotein inhibitors. Meanwhile, only Sildenafil acts as an inhibitor for class 2 inhibitors. All the ligands have < 0.45 VD value meanwhile Sildenafil displayed high VD. All selected ligands from the current study display BBB permeability except for Sildenafil. In this study, different models of isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) were used to predict the Cytochrome P450 inhibitory capabilities of a compound. It was found that none of the ligands and Sildenafil show inhibitory effects on CYP1A2, CYP1A2, CYP2C19, CYP2C9. CYP2D6 & CYP3A4 are 2 isoforms of Cytochrome P450 substrate which are used to assess the metabolism of the compound. The current study shows that all the ligands and the Sildenafil were capable of acting as CYP3A4 substrates.

The selected ligands showed no toxicity in the current study. Toxicity studies, like AMES toxicity, skin sensitivity, cardiotoxicity, and hERG I and II inhibition revealed negative results except for Sildenafil which displayed hERG II inhibition hepatotoxicity.

Table 2
ADMET analysis of top three ligands compared with Sildenafil (control)

Properties	Diosgenin,dehydro	Ruscogenin	Hecogenin	Sildenafil (Control)
Absorption				
Water solubility (log mol/L)	-5.903	-5.096	-5.028	-3.07
Caco2 permeability (log Papp in 10 ⁻⁶ cm/s)	1.282	1.264	1.432	0.135
Intestinal absorption (human) (% Absorbed)	98.678	96.811	96.696	81.256
Skin Permeability (log Kp)	-3.373	-4.014	-3.506	-2.735
P-glycoprotein substrate	No	Yes	No	Yes
P-glycoprotein I inhibitor	Yes	Yes	Yes	Yes
P-glycoprotein II inhibitor	Yes	Yes	Yes	No
Distribution				
VDss (human) (log L/kg)	0.314	0.005	0.221	1.091
Fraction unbound (human) (Fu)	0	0.041	0.012	0.172
BBB permeability (log BB)	0.275	0.119	-0.043	-1.416
CNS permeability (log PS)	-2.925	-1.677	-1.443	-3.531
Metabolism				
CYP2D6 substrate	No	No	No	No
CYP3A4 substrate	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	No	No	No	No
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	Yes
Excretion				
Total Clearance (log ml/min/kg)	0.243	0.375	0.315	0.251
Renal OCT2 substrate	No	Yes	Yes	No
Toxicity				

Properties	Diosgenin,dehydro	Ruscogenin	Hecogenin	Sildenafil (Control)
Absorption				
AMES toxicity	No	No	No	No
Max. tolerated dose (human) (log mg/kg/day)	-0.609	-0.669	-0.615	0.209
hERG I inhibitor	No	No	No	No
hERG II inhibitor	No	No	No	Yes
Oral Rat Acute Toxicity (LD50) (mol/kg)	1.881	2.062	2.028	2.655
Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	1.296	1.268	1.133	1.965
Hepatotoxicity	No	No	No	Yes
Skin Sensitisation	No	No	No	No
<i>T.Pyriformis</i> toxicity (log ug/L)	0.36	0.351	0.339	0.286
Minnow toxicity (log mM)	0.118	0.787	1.192	0.889
Molecular Properties				
Molecular Weight	396.615	430.629	430.629	474.587
LogP	6.5191	4.6847	4.9728	1.6109
nRot	0	0	0	7
nHA	2	4	4	8
nHD	0	2	1	1
TPSA	177.041	187.319	187.376	192.781
Lipinski violation	No	No	No	No

ADMET parameters of identified phytochemicals from *T. terrestris* were analysed using the pkCSM tool. Abbreviations: VDss- steady-state volume of distribution, BBB permeability (log BB)- Blood–Brain Barrier permeability, CNS permeability (log PS)- Central Nervous system permeability, Renal OCT2 substrate- Renal Organic Cation Transporter 2 substrate, *T.Pyriformis*- *Tetrahymena pyriformis*, LogP- log of the partition coefficient, nRot- number of rotatable bonds, nHA- number of Hydrogen-bond Acceptors, nHD- number of Hydrogen-bond Donors, TPSA- Topological polar surface area.

A general guide for ADMET properties in pkCSM tool is as follows, Caco2 permeability > 0.90, Intestinal absorption > 30%, Skin permeability LogKp>-2.5, VDss should lie between – 0.15- to 0.45, BBB

permeability ranging between (-1)- to 0.3, CNS permeability should lie between (-3)- to (-2), *T.pyrifomis* toxicity should be $<-0.5 \log \mu\text{g/L}$, Minnow toxicity should be $\text{LC}_{50} > -0.3$.

3.3 Molecular Dynamic Simulations

All simulations were performed with post-docking structures. Our analysis of the MD simulation focuses mainly on the plasticity of the protein and binding sites observed. The binding of small molecules to the PDE5 catalytic site could instigate communication across the structure of the protein and influence the catalytic domain's function while reducing its affinity for cGMP.

3.3.1 Root Mean Square Deviation (RMSD)

RMSD is essential in measuring the MD trajectory equilibration and predicting the stability of complicated systems throughout the simulation. The RMSD values for the PDE5-Sildenafil complex, PDE5-Hecogenin complex, PDE5-Diosgenin,dehydro complex, PDE5-Ruscogenin complex and PDE5 free protein are shown in Fig. 3 (A). The RMSD plot of the Ruscogenin complex shows the largest deviation from the initial docked structure $\sim 9 \text{ \AA}$. Despite this shift, the compound continues to interact with the critical residues at the active site, as identified in the subsequent analyses. PDE5-Sildenafil remains relatively stable after 150 ns around $\sim 4 \text{ \AA}$. PDE5-Hecogenin complex, PDE5-Diosgenin,dehydro complex, and PDE5 free protein reveal the lowest RMSD values of $\sim 2.5 \text{ \AA}$.

3.3.2 Root Mean Square Fluctuation (RMSF)

RMSF is a crucial metric to exhibit the stability and flexibility of complex systems. The RMSF values for individual residues in the protein were plotted for each of the protein-ligand complexes given in Fig. 3 (B). The fluctuations predominantly peak at two locations, the 650–700 amino acid region, and the 775–825 amino acid region. The 650–700 amino acid region of the protein shows more fluctuation when bound with Hecogenin, Diosgenin,dehydro in comparison to the ligand-free PDE5 protein. PDE5-bound Sildenafil and Ruscogenin show the least fluctuation in the mentioned region of the protein. All of the ligand-bound structures display large fluctuations in the 775–825 amino acid region with respect to the ligand-free protein.

3.3.3 Radius of gyration (Rg)

The radius of gyration (Rg) is the root mean square distance between the protein's atoms and the rotation axis is in the range and determines the protein structure compactness. PDE5 free protein and PDE5-Sildenafil complexes given in Fig. 3 (C) show reduced Rg ranging from $\sim 19.6\text{--}20.4 \text{ \AA}$. PDE5-Ruscogenin complex's Rg ranges from $\sim 19.7\text{--}20.6 \text{ \AA}$ and converges towards $20.1 \text{ \AA}$. PDE5 bound Hecogenin complex and Diosgenin,dehydro complex show the most variation in Rg, ranging from $\sim 19.7\text{--}20.8 \text{ \AA}$; the Rg of Hecogenin complex and Diosgenin,dehydro complex converges to $20.1 \text{ \AA}$ and $20.5 \text{ \AA}$ respectively, towards the end of the simulation.

3.3.4 Solvent Accessible Surface Area (SASA)

Throughout the 200 ns simulation, the Solvent Accessible Surface Area (SASA) for the PDE5 free protein exhibited a range between 15477.6 Å² – 18203.6 Å², with an average of 16585.9 Å². Sildenafil bound PDE5 complex's SASA ranged between 15641.5 Å² -18111.6 Å² with an average SASA of 16952.6 Å². PDE5 bound Hecogenin, Diosgenin, dehydro and Ruscogenin SASA values ranged 15422.2 Å² -18780.3 Å², 15741.8 Å² -18068.4 Å² and 15797.5 Å² -18249.3 Å² with individual average 17497.1 Å², 17154.1 Å² and 17172.1 Å² respectively as visualized in Fig. 3 (D).

3.3.5 Hydrogen Bond Analysis (H-bond analysis)

Hydrogen bond analysis of the MD simulations identified the residues that showed H-bond interactions with the ligand during the course of the 200 ns simulation as seen in Fig. 3 (E). Sildenafil showed robust H-bond interaction with ASN662, and intermittent interaction with ASN661; feeble interaction was also observed with TYR612, TYR664, THR723, LEU725 and GLN789. Hecogenin showed the greatest H-bond interaction with ASN662, limited interaction with GLN817 and TYR612, and the least interaction with TYR664, LEU725 and GLN775. Ruscogenin displayed the least H-bond interaction, exhibiting feeble interaction with ASN661 and ASN662. Diosgenin, dehydro showed interspersed H-bond interaction with GLN817 for the first 75 ns which re-emerged at ~ 200 ns.

3.5.6 Molecular mechanics with Poisson-Boltzmann and surface area solvation (MMPBSA)

MMPBSA provides an aggregate score of the binding free energy of the protein-ligand complex by considering various energy terms⁵⁴. The Enthalpy of binding calculated and represented in Table 3 and Fig. 4. denoted the PDE5-Diosgenin, dehydro complex to have the highest Enthalpy contribution followed by Sildenafil, Hecogenin, and Ruscogenin complexes. We also observe in the $-T\Delta S$ component which represents the conformational entropy after ligand binding, the least entropic contribution is noted in the PDE5-Diosgenin, dehydro complex followed by Hecogenin, Sildenafil, and Ruscogenin complexes. By assessing the contributions from individual residues in energy terms by free energy decomposition analyses shown in Fig. 4, we found that the most consistent contributions were attributed to VAL782, PHE786, and PHE820 in all the protein-ligand complexes.

The overall free binding energy for each complex was calculated and is shown in Fig. 4. As a result of the large enthalpy component and low entropic term, the computed value of the effective free energy was greatest in the case of the PDE5-Diosgenin, dehydro complex followed by Hecogenin, Sildenafil and Ruscogenin complexes.

Table 3
MMPBSA energy for the top three protein-ligand complexes and Sildenafil (control).

S. No.	Protein-ligand Complex	ΔG_{bind} (kcal/mol)
1.	PDE5-Sildenafil	-9.1 ± 6.22
2.	PDE5-Ruscogenin	-9.05 ± 5.16
3.	PDE5-Hecogenin	-14.11 ± 5.33
4.	PDE5-Diosgenin,dehydro	-19.99 ± 5.99

3.3.6 PCA (Principal component analysis)

PCA is a technique used in the analysis of datasets containing a high number of dimensions or features per observation, PCA allows us to interpret the data while preserving the maximum amount of information, hence facilitating the visualization of multidimensional data. PCA is performed by reducing the dimension of the dataset keeping the critical information that is characterized by the eigenvectors. The scree plot for Eigenvalue rank vs proportion of variance for each of the complexes shows that the top 20 principal components were able to capture more than 80% of the variance of conformation fluctuations observed during the molecular dynamic simulation of the protein-ligand complexes while only up to 75% for that of the free-protein was observed.

The first Principal component accounted for 52.9%, 44.7%, and 37% of the variance in the motion observed in the trajectories of Ruscogenin, Hecogenin, Diosgenin,dehydro with covariances of 83.6%, 87.6%, and 81.4% respectively, while PCs of Sildenafil and the free-protein accounted for 35.9% and 25.5%, with a covariance of 80.5% and 75.3%. The increased covariance captured by the fewer principal components suggest that the drug binding reduces the conformational flexibility of the protein.

Considering the first three principal components as they account for most of the variance, they were projected along the eigenvector direction to produce a scatterplot to depict how the protein-ligand system evolves over time. We observe a clear distinction in the initial conformation of the protein and final conformation with a well-defined intermediate region indicated by the discrete regions defined by continuous color representation (from blue to white to red), most prominently noted in Hecogenin and Ruscogenin as represented in Fig. 5.

4. Discussion

Erectile dysfunction (ED) is a common condition affecting men worldwide, regardless of age, and has been a concern for centuries, with the continuous urge to find remedies dating back to ancient times^{31,68}. The prevalence of ED in men under 40 years old ranges from 1–10%, as per a recent analysis by the International Consultation Committee for Sexual Medicine, suggesting its occurrence among younger men⁶⁹. It's a significant concern as, despite their vigor and physical health, ED can affect the quality of

life and intimate relationships. This highlights the complex nature of ED, which can stem from a various factor beyond just physical fitness, including psychological stress, relationship issues, lifestyle habits, and underlying medical conditions. Lifestyle disorders such as obesity and hypercholesterolemia are also reported as major causative factors.^{70,71} ED can arise from either medical conditions (organic) or situational/psychological factors (non-organic). Organic ED results from physical abnormalities affecting penile function, typically involving nerves, hormones, blood vessels, tissues, or muscles, while non-organic ED is often linked to mental health concerns like anxiety or relationship difficulties. Despite the availability of medications for treating erectile dysfunction, a significant percentage of the population avoids these drugs due to inefficacy or adverse side effects. This indicates a need for more reliable treatment strategies that offer better efficacy, pharmacokinetics, and fewer side effects⁷².

The most commonly prescribed PDE5 inhibitor is Sildenafil, known by its chemical name 5-[2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, stands as the inaugural active pharmaceutical ingredient (API) structure to be systematically designed through computational drug-design methodologies, with specificity to inhibit PDE5⁸. However, its usage has been linked to numerous adverse effects, including reported fatalities, syncope, myocardial infarctions, strokes, and cardiac arrhythmias⁷³. The post-market surveillance data submitted to the FDA revealed concerning trends shortly after its introduction and within eight months, reports linked Sildenafil usage to 130 confirmed deaths and 112 unverified fatalities⁷⁴. Subsequently, after 13 months on the market, a total of 1,473 major adverse events were reported, including 522 deaths⁷⁵. Despite its limited approved use, Sildenafil has gained popularity as a recreational drug among young adults without medical necessity could lead to overdosing. The primary risk of using Sildenafil without medical supervision is the potential for dangerous interactions with other medications, such as nitrates, leading to severe drops in blood pressure and risking heart attack or stroke²⁰. Additionally, Sildenafil misuse can result in long-term health issues like vision and hearing loss, as well as painful conditions like priapism⁷⁶. Psychological dependence is also a concern, as recreational users may rely on Sildenafil to achieve sufficient erections, potentially exacerbating health issues⁷⁷.

Plant-based treatments and phytochemicals could offer a promising alternative for treating ED, particularly due to their potential for fewer side effects compared to synthetic drugs⁷⁸. Various natural compounds from diverse chemical classes have demonstrated efficacy against ED, with claims of inhibitory effects on PDE5^{79,80}. Recent studies have found that the most common active ingredients in these supplements include ginseng, L-arginine, tongkat ali, horny goat weed, tribulus, maca, muira puama, zinc, saw palmetto, and fenugreek⁸¹. Studies have been carried out on phytochemicals present in other plants with aphrodisiac properties such as *Mimosa pudica* Linn., *Mirabilis jalapa* L., and *Withania somnifera* (Ashwagandha or Indian ginseng) holding promise for addressing male sexual dysfunction targeting PDE5⁸²⁻⁸⁵. Icariin is a well-known compound from *E. sagittatum*, also known as horny goat weed, believed to be the most effective in the treatment of ED^{86,87}. It was found that oral intake of Icariin (1 and 5 mg/kg) increased the expression of NOS isoforms and inhibited PDE5, whereas dosage of 50

and 100 mg/kg for 35 days significantly increased testosterone levels in adult male Sprague-Dawley rats⁸⁶. However, more studies were needed to refine those potent phytochemicals either as a single constituent or as a formulation to use as a safer drug.

The genus *Tribulus* is emblematic of phytochemicals with aphrodisiac properties in ethnobotanical surveys and folklore medicinal studies^{88,89}. Various parts of this plant contain medicinally important chemical constituents, including flavonoids, flavonol glycosides, steroidal saponins, and alkaloids^{32,90}. The active ingredients present in the extracts, such as Dioscin, Diosgenin, and Protodioscin, are steroidal molecules believed to increase sex hormone levels⁹¹. Preparations derived from the saponin fraction of *Tribulus terrestris* are utilized for treating infertility and libido disorders in both men and women⁹². Important formulations composed of *T. terrestris* extract are Gokshuradi Guggulu, Trikantak Ghruta, Drakshadi Choorna, Rasayana Choorna, Gokshuradi Kwatha, Dashamoola Kwatha³². *T. terrestris* shows promise in regulating various pathways that are crucial for cardiovascular, hormonal, and sexual health. Its potential effects include enhancing NO production, activating antioxidant pathways (Nrf2/HO-1), inhibiting inflammatory pathways (NF- κ B), boosting serum testosterone and DHEAS levels, and inhibiting PDE5^{93,94}. The effect of *T. terrestris* phytochemicals on PDE5 inhibition has not yet been studied. Therefore, considering its aphrodisiac potential and the role of PDE5 in the etiology of erectile dysfunction, we conducted a screening of phytochemicals from *T. terrestris*. Using *in-silico* tools, we assessed their binding affinity to the target protein PDE5 and evaluated their pharmacokinetic and physicochemical properties. We aimed to investigate whether these phytochemicals have inhibitory effects on PDE5, which could contribute to maintaining erections for satisfactory sexual performance.

The hypothesis derived from our study suggests that 13 phytochemicals from *T. terrestris* could potentially inhibit the PDE5 enzyme as they showed higher binding affinity than the control drug, Sildenafil. This inhibition could lead to increased nitric oxide release and elevated cGMP levels in the corpus cavernosum inducing smooth muscle relaxation, facilitating blood flow, and prolonging penile erection⁹⁵. From the top 13 phytochemicals selected from *T. terrestris*, three promising molecules, Diosgenin, dehydro, Ruscogenin, identified from aerial parts, and Hecogenin, identified from aerial part, flower, fruit, leaf, root, and stem were selected based on their binding affinities and strong interactions with the PDE5 enzyme. Phytochemicals like Diosgenin, Stigmasterol, Quercetin, Kaempferol, and Astragalin have already been studied for their proven efficacy in treating sexual dysfunctionalities^{78,96,97}. Here we report a phytochemical found in *T. terrestris*, Diosgenin, dehydro, a derivative of Diosgenin having a better dock score as well as pharmacokinetic properties than Diosgenin (Supplementary table 2). Ruscogenin is a major steroidal saponin belonging to triterpenoids and is also found in *Cordyline australis* and *Ophiopogon intermedius*. Both Ruscogenin and Diosgenin, dehydro show similar binding affinities to the target protein, however Diosgenin, dehydro is poorly soluble. The three phytochemicals exhibit favorable absorption characteristics, high intestinal absorption rates, and low skin permeability, indicating efficient uptake and distribution. Sildenafil, though widely used, has been associated with several adverse effects, including hepatotoxicity and hERG II channel inhibition. Despite Ruscogenin's

interaction with P-glycoprotein, the overall pharmacokinetic profiles suggest improved safety and tolerability compared to Sildenafil.

The Molecular dynamics analysis of the protein-ligand complex was carried out to understand the structural and dynamic nature of the top compounds within the active region of PDE5⁵³. RMSD analysis indicated that the Hecogenin and Diosgenin,dehydro complex exhibited the least fluctuation, comparable to the RMSD plot of the free protein and lower than the standard drug Sildenafil. The RMSF plot revealed significant fluctuation in the 650–700 and 775–825 amino acid regions in the PDE5 catalytic domain which is crucial for the stability of the complex. Rg analysis investigated the influence of interactions between PDE5 and its ligands on the protein's conformational structure. Diosgenin,dehydro exhibited the lowest Rg values throughout the simulation compared to the control drug, indicating a compact structural configuration for PDE5, with minimal fluctuations and no unfolding events. We analyzed the results of SASA values throughout the simulation to examine the folding and unfolding behaviors of PDE5, both in its free state and when bound to ligands. SASA values offer insights into the degree of solvent exposure for individual amino acids within the protein. These values closely correspond with the trends observed in Rg over the 200 ns simulation trajectory. A slight reduction in SASA values upon ligand binding suggests tighter protein packing, especially notable with Diosgenin,dehydro and Sildenafil. Hydrogen bond analysis of Sildenafil revealed distinct binding patterns among the inhibitors at the catalytic site of PDE5 with key interactions with ASN662 and ASN661, along with feeble contacts involving TYR612, TYR664, THR723, LEU725, and GLN789. Hecogenin exhibited strong bonding with ASN662 and limited interactions with GLN817 and TYR612, while Diosgenin,dehydro displayed intermittent bonding with GLN817 within the first 75 ns and again near 200 ns, which is an important amino acid residue known to stabilize the inhibitor Tadalafil^{51,98}. Tadalafil is known to have a higher affinity towards PDE5 than PDE6 making it more selective than Sildenafil and mutation studies such as Q817G demonstrate the significance of GLN817 interaction in determining PDE5 affinity for cGMP as well as several inhibitors¹³. However, Tadalafil is reported to block hERG1 (human Ether-à-go-go-Related Gene) potassium (K⁺) channels in a concentration-dependent manner, which is critical as its inhibition can lead to prolonged QT intervals on an electrocardiogram, increasing the risk of potentially fatal arrhythmias, such as Torsades de Pointes^{99,100}. In addition, Tadalafil is a dual inhibitor of PDE5 and PDE11 enzymes, which is thought to be the reason for back and muscle pain (myalgia) during treatment, as PDE11 enzymes are abundantly found in skeletal muscle cells²⁴. Moreover, binding free energy per residue calculations highlighted the positive contributions of residues such as VAL782, PHE786, PHE820, ILE768, LEU725, and ILE813 to the stability of the complexes. PCA plots indicated the least variation in the Diosgenin,dehydro complex, correlating with the energy terms obtained from MMPBSA analysis, suggesting that the binding of Diosgenin,dehydro stabilizes the PDE5-inhibitor complex, thereby indicating better inhibition of PDE5. The energetic content of PDE5 in the presence of *T. terrestris* compounds as inhibitors focusing on the inter- and intramolecular forces contributing to the binding interactions. These forces include hydrogen bonding, electrostatic interactions, van der Waals forces, and hydrophobic interactions, which collectively stabilize the inhibitor-enzyme complex¹⁰¹. We have not calculated the intramolecular forces directly but we have calculated the effect that intramolecular forces

can have on SASA and Rg of the PDE5 protein as a free protein and ligand-bound complex. These findings contribute to the ongoing efforts to develop novel and safer treatments for ED, particularly addressing the limitations of currently available drugs.

5. Conclusion

Our study aimed to identify potent bioactive molecules with fewer side effects for treating erectile dysfunction (ED) through molecular docking and simulation studies. Despite the popularity of synthetic drugs, particularly PDE5 inhibitors, the adverse side effects and inefficiency of the drug remain a major challenge in ED treatments. A potential alternative for this challenge could be employing plants with aphrodisiac properties such as *T. terrestris* which might have lesser side effects. Through screening of phytochemicals from *T. terrestris*, this study assessed their binding affinity to the target protein PDE5 and evaluated their pharmacokinetics and physicochemical properties. The results suggest that phytochemicals Diosgenin, dehydro and Hecogenin from *T. terrestris* may inhibit the PDE5 enzyme, leading to increased nitric oxide release and elevated cGMP levels, facilitating smooth muscle relaxation and penile erection. As a consequence of our study, developing a new drug that combines the exceptional absorptive properties of Ruscogenin with the strong binding interactions of Diosgenin, dehydro shows promise in creating new and effective interventions against ED. Given the prevalence of ED among young men and concerns regarding the adverse effects of conventional treatments, exploring natural remedies presents a compelling avenue for safer and more effective treatment options. However, this is a computational study which further requires *in vitro* and *in-vivo* experimental validation. The study underscores the potency of plant-derived compounds as promising candidates for ED management, with implications for improving patient outcomes and quality of life.

Declarations

CRedit authorship contribution statement

Riya Vishwakarma: Writing- original draft, Formal analysis, Visualization, Validation, Methodology, **Abel John Koshy:** Methodology, Software, **Haritha Kalath:** Formal analysis, Visualization, Validation, Data curation, Methodology, **Krishnapriya Ramakrishnan:** Writing- review & editing, **Anish John:** Writing- review & editing, **Sowmya Soman:** Writing- review & editing, **Rajesh Raju:** Writing- review & editing, Supervision, Project administration, **Niyas Rehman:** Writing- original draft, Writing- review & editing, Conceptualization, Investigation, Project administration, **Amjesh Revikumar:** Writing- review & editing, Conceptualization, Investigation, Methodology, Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

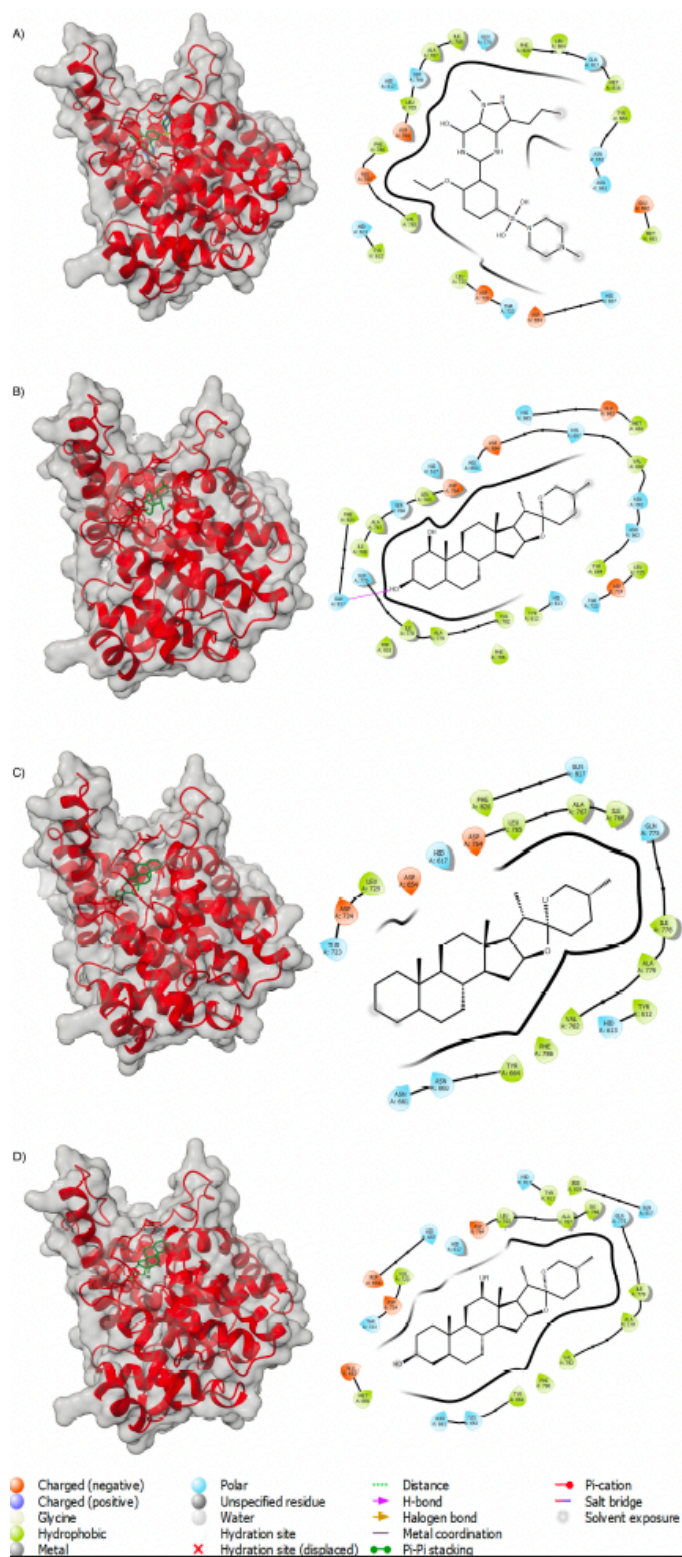


Figure 1

Molecular docking poses and ligand interaction diagrams of PDE5 with (A) Sildenafil (control), (B) Ruscogenin, (C) Diosgenin, dehydro, and (D) Hecogenin are displayed. The types of interactions are represented at the bottom

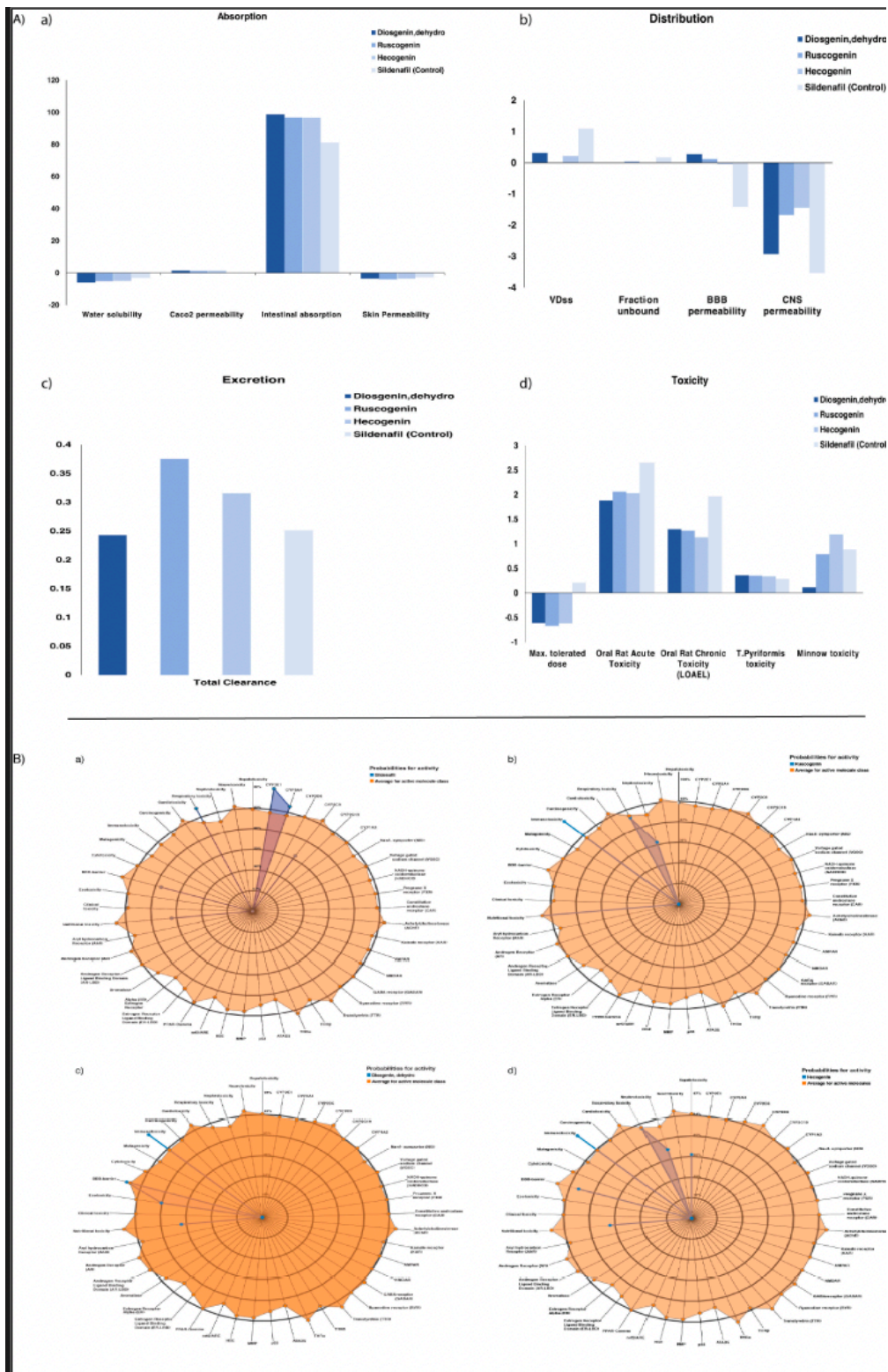


Figure 2

Comparative ADMET Analysis of Phytochemicals and Sildenafil (control).

A. Bar plots depict a comparative assessment of the (a) Absorption, (b) Distribution, (c) Excretion, and (d) Toxicity profiles of the top three phytochemicals in comparison to Sildenafil (control).

B. Toxicity results are represented using radar plots for (a) Sildenafil (control), (b) Ruscogenin, (c) Diosgenin,dehydro, and (d) Hecogenin. According to the ProTox-II classification scheme, orange dots/lines indicate confidence levels of positive toxicity results, computed from the training set data for each model, relative to the average of each class

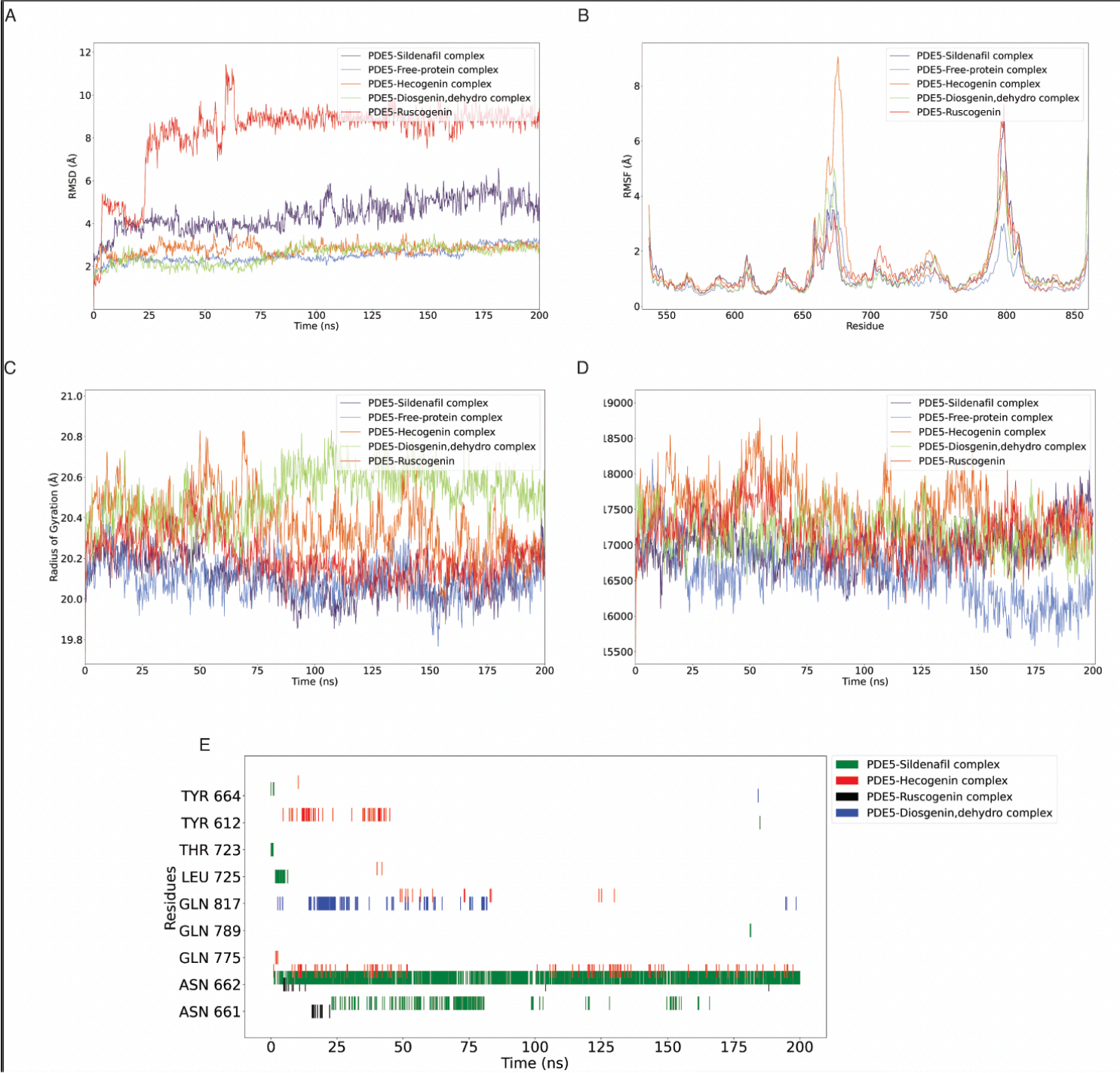


Figure 3

Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of gyration plot, Solvent accessible surface area (SASA), and Hydrogen bond analysis for the top three phytochemicals and Sildenafil (control) in complex with protein PDE5 over 200 ns MD simulation. A) RMSD versus time plot, B) RMSF trajectories for residues C) Graph depicting Radius of Gyration of the protein D) Solvent

accessible surface area plot E) Hydrogen bond analysis for the residue's contribution throughout the 200 ns MD simulation

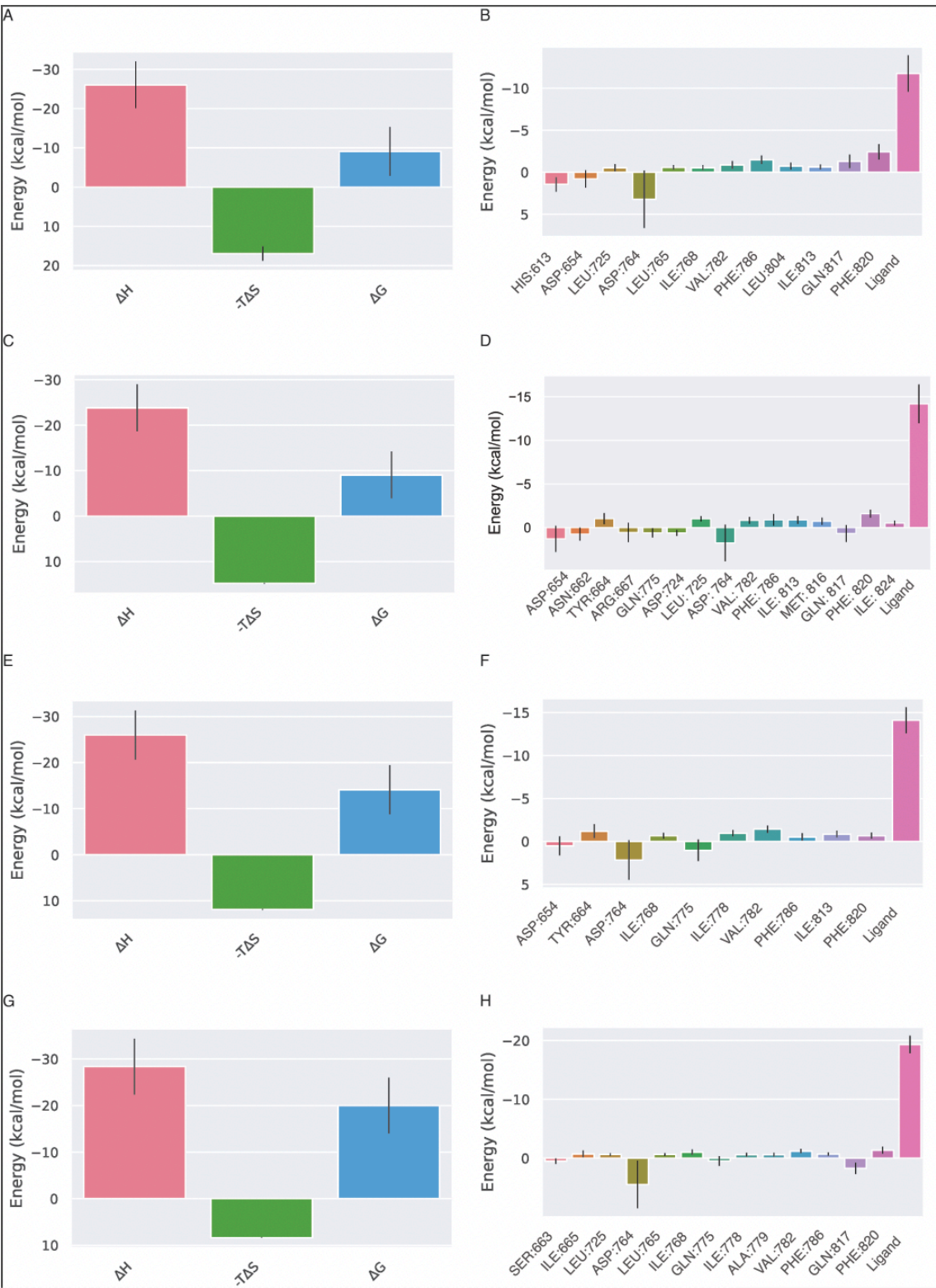


Figure 4

End-state free energy calculations of the MD simulations. Panels (A), (C), (E), and (G) represent the total binding free energy calculations, while panels (B), (D), (F), and (H) depict the corresponding MMPBSA

per-residue decomposition energies for the following complexes: PDE5-Sildenafil (A, B), PDE5-Ruscogenin (C, D), PDE5-Hecogenin (E, F), and PDE5-Diosgenin-Dehydro (G, H), respectively.

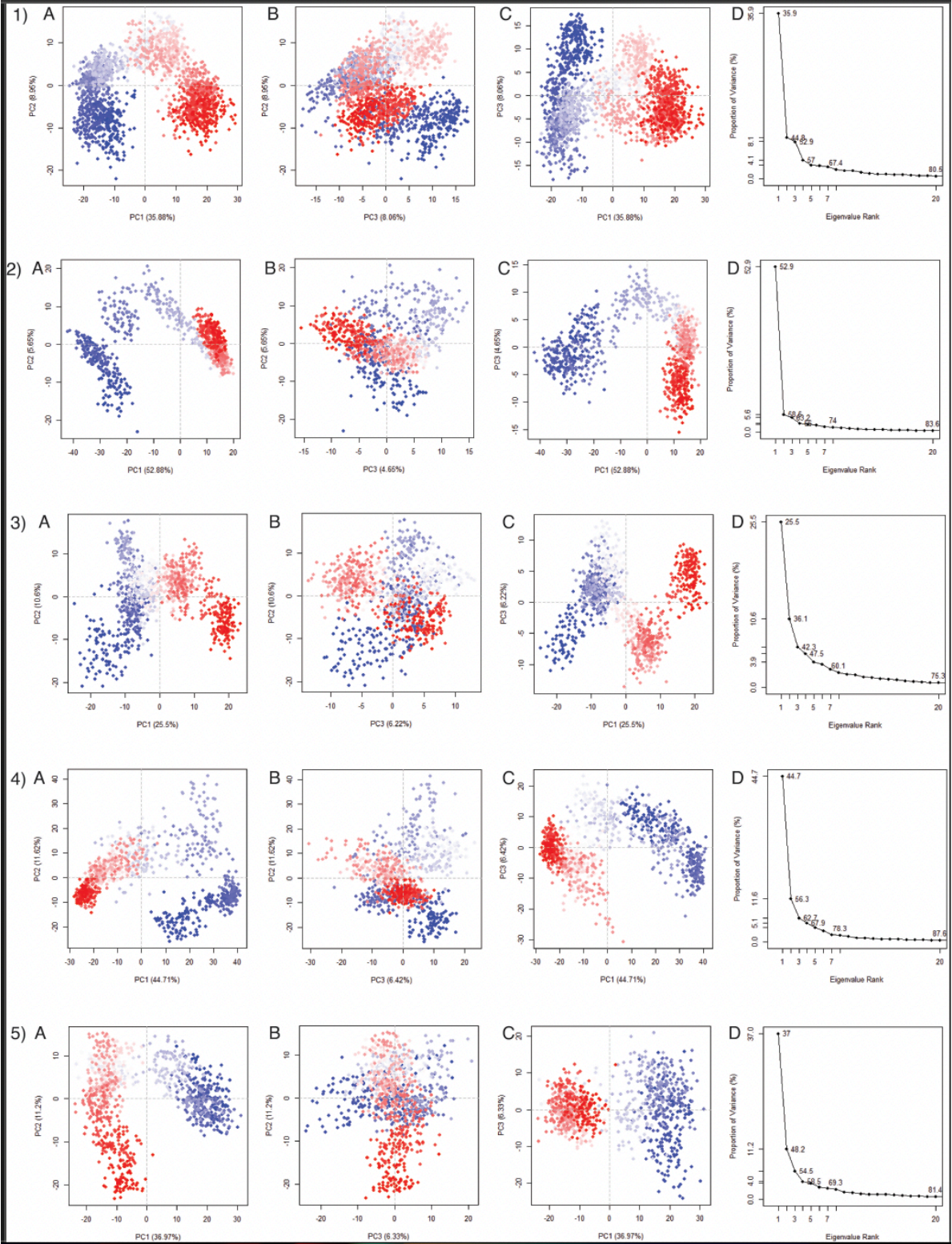


Figure 5

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