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Aphrodisiac Potential of Hibiscus sabdariffa: Phosphodiesterse-5 Inhibition and Sexual Behavior Study Using in Silico and in Vivo Models

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ABSTRACT:

Background: Driven by in vitro, in silico, and in vivo studies, the current work investigates the therapeutic potential of Hibiscus sabdariffa L. flower extract in modulating male sexual function through phosphodiesterase-5 (PDE-5) inhibition. Sexual dysfunction cause is also a s metabolic disorder. The bioactive components including gossypitrin, luteolin, hibiscetin-3-glucoside, and delphinidin-3-sambubioside, were found using molecular docking studies to have beneficial binding affinities for PDE-5 that were either higher than or equivalent to those of the reference with sildenafil. The stability and potent intermolecular interactions of these compounds within the PDE-5 active site have been further demonstrated by molecular dynamics (MD) simulation, indicating their potential pharmacological significance.

Methods: To assess the extract's impact on male genitals, in vivo tests were carried out on rat's models. at doses of 100, 200, and 400 mg/kg, the animals were split into five groups: Measurements of parameters like mount frequency, mount latency, and intromission delay showed that sexual performance improved in a dose-dependent manner.

Results: The behavioral results were supported by biochemical analyses that showed a substantial rise in serum testosterone levels and nitric oxide concentration. Furthermore, the extract's PDE-5 inhibitory efficacy validated its mechanism of action in improving erectile function.

Conclusions: When combined, the results show that Hibiscus sabdariffa flower extract is a potential natural substitute for enhancing male sexual health via a variety of multimodal mechanisms, such as nitric oxide modulation, testosterone regulation, and PDE-5 inhibition. Its therapeutic potential is highlighted by this integrated method that combines computational predictions with experimental confirmation, opening the door for the eventual development of phytotherapeutics that target erectile dysfunction.

INTRODUCTION:

Hibiscus sabdariffa L. (Malvaceae), also known as roselle or "sour tea," is a medicinal and dietary plant native to Africa that is now grown globally. Its calyces are commonly used in beverages and meals, and they are prized in traditional medicine for treating hypertension, liver problems, diabetes, and infections, demonstrating their broad pharmacological importance. [1,2]. *H. sabdariffa* can be distinguished pharmacologically by its red calyces, mucilaginous polysaccharides, and anthocyanin colors.

Macroscopic, microscopic, and physicochemical characteristics aid in quality assessment, whereas rising nutraceutical consumption emphasizes the requirement for dependable indicators to avoid falsification and assure continuous bioactivity [3,4]. Phytochemical studies have shown a diverse range of secondary metabolites in roselle. Anthocyanins (delphinidin-3-sambubioside, cyanidin-3-sambubioside), flavonoids (luteolin, quercetin, gossypetin, hibiscetin derivatives), and phenolic acids (protocatechuic, chlorogenic, caffeic, and ferulic acids) are among the most important bioactive components. Organic acids including hibiscus acid, citric acid, and malic acid contribute to its sour flavor and have diuretic and antihypertensive properties. Sterols, polysaccharides, vitamin C, and vital minerals are among the additional components identified. The presence of antioxidant phytoconstituents provides a solid molecular foundation for its diverse therapeutic properties [5-7].

A rising collection of experimental evidence shows that *H. sabdariffa* has a variety of medicinal applications. In vitro and in vivo research have confirmed its antioxidant, anti-inflammatory, antibacterial, hepatoprotective, nephroprotective, antidiabetic, anti-obesity, and anticancer effects. Anthocyanins and hibiscus acid reduce angiotensin-converting enzyme (ACE), cause vasodilation, and enhance lipid metabolism. The results obtained lend credence to the conventional use of hibiscus tea to manage high blood pressure and dyslipidaemia. Also, protocatechuic acid and other phenolics have been found to have cytoprotective and chemo preventive effects by regulating oxidative stress and apoptotic pathways [8-10].

Clinical trials have shown that hibiscus tea or standardized extracts can dramatically lower systolic and diastolic blood pressure, with efficacy comparable to first-line antihypertensive medications. Several studies suggest benefits in lipid profile and glycemic management, while results vary depending on dosage and formulation. Overall, hibiscus is well taken with just minimal gastrointestinal symptoms, indicating that it is safe for long-term use [11,12]. Delphinidin-3-sambubioside was extracted from *Hibiscus sabdariffa* L. and confirmed hypolipidemic and antihypertensive effects in rat's animal model [13]. In vitro and in silico studies show that hibiscus acid inhibits the angiotensin-converting enzyme (ACE) [14]. *H. sabdariffa* extract's diuretic and antihypertensive properties are due to its capacity to impede angiotensin-converting enzyme (ACE) and other enzymes implicated in the onset of hypertension [15].

Erectile dysfunction is often linked to metabolic illnesses, particularly diabetes mellitus. It is believed that 32% of people with type 1 diabetes and 46% of people with type 2 diabetes have ED, with some estimates suggesting rates as high as 75%. As one ages, the risk becomes even more [16]. Low libido and decreased sexual satisfaction have also been linked to diabetes mellitus, obesity, and dyslipidaemia [17].

Computational chemistry has become an essential instrument for bridging the gap between theoretical ideas and experimental results. It enables accurate study of chemical structures, reactivity, and interactions at the atomic and molecular levels through the use of quantum mechanics, molecular modelling, and simulation techniques. This method offers predicted insights into biological behaviour while drastically lowering the expense, time, and ethical issues related to field research [18,19].

MATERIALS AND METHODS:

Chemicals: All chemicals were of analytical grade and purchased from Sigma-Aldrich

In silico study:

Delphinidin-3-sambubioside, hibiscetin-3-glucoside, luteolin, and gossypetin have been previously reported by researchers to exhibit potential aphrodisiac activity [20,21].

Molecular docking and ADMET:

By the computational approach these studies have performed, followed four major steps, (a) Protein Preparation and Grid Generation—The structure of human serum albumin bound to Indomethacin [PDB ID:2H42] was retrieved from the Protein Data Bank and prepared for docking, (b) Ligand Preparation – Ligands were processed with LigPrep using the OPLS-5 force field for geometric minimization, (c) Molecular Docking—The GLIDE docking module was employed for ligand-receptor interaction studies and (d) MM-GBSA Analysis – Prime/MM-GBSA was applied to estimate the relative binding free energy of the ligands [22,23].

Molecular Dynamics (MD) Simulation:

Molecular dynamics (MD) simulations were conducted with the GROMACS 2022.2 package to evaluate the dynamic stability of PDE5–ligand complexes. The simulation workflow involved the following sequential steps.

Preparation of the Enzyme-Ligand Complexes

The three-dimensional (3D) structures of the protein-ligand complexes were initially prepared and exported in .pdb format using PyMOL. Protein topologies were generated using the pdb2gmx module with the CHARMM27 all-atom force field. Ligand topologies were constructed via the Swiss Param server, which is compatible with the CHARMM force field parameters [24,25].

System Setup and Equilibration

Every combination was solvated employing the TIP3P water model after being put in a cubic simulation box with a minimum of 1 nm separating the protein surface from the box edge [26]. In every direction, periodic boundary conditions were used. Na+ counterions were added in the proper quantity to electrically neutralize the systems. The steepest descent approach was used for 50,000 steps of energy minimization in order to optimize initial shape and eliminate steric conflicts.

A 100 ps NVT ensemble (constant number, volume, temperature) simulation at 300 K and a 100 ps NPT ensemble (constant number, pressure, temperature) simulation to equilibrate pressure at 1 bar was the two equilibration phases that followed minimization. Time integration was performed using the leap-frog integrator, and the protein, ligand, solvent, and ions were subjected to individual temperature coupling using the Berendsen thermostat (coupling constant = 0.1 ps) and Berendsen barostat (coupling constant = 2.0 ps) [27, 28].

After that, production MD simulations were run for 100 ns at 300 K and 1 bar with NPT circumstances. The LINCS algorithm was used to apply bond limitations, and the Parrinello-Rahman barostat was used to control the pressure coupling with a time constant of 1 ps [29]. The Particle Mesh Ewald (PME) approach was used for addressing long-range electrostatics [30], and both van der Waals and Coulomb interactions were cutoff at 1.2 nm.

Trajectory Visualization and Data Analysis

Version 1.9.2 of Visual Molecular Dynamics (VMD) was used to display trajectory files [31]. Hero MD Analysis [32], a specially designed in-house program for extracting structural measures, and Xmgrace version 5.1.25 for statistical and graphical presentation were used for quantitative studies.

2.4 Plant extract: The acquired fruits were first divided into four tiny fragments and left to dry outdoors at room temperature. After being further diced using a chopper, the fruits were dried in an oven set to 40–45 °C for two to three days until their weight remained consistent. The resulting dried materials were ground into a coarse powder using a grinder. Petroleum ether (40–60 °C) was used to defatten the coarse powder, and it was macerated for 72 hours with 80% ethanol. A rotary evaporator was then used to concentrate the extract until it was dry at 40 °C after it had been filtered through Whatman filter paper. For later usage, it was stored in the refrigerator [33,34].

Animal study

Healthy albino rats weighing 135-140 ± 5 g were purchased from the animal facility the Institutional Animal Ethics Committee (IAEC) gave its approval (SCBR-439/2025). For a week, each animal was housed separately in polypropylene cages with a 12-hour light-dark cycle, regulated temperature (21–27 °C), and relative humidity. Animals were given a regular pellet meal and unlimited water during this acclimation phase.

Upon completion of acclimatization, the rats were assigned to five experimental groups (n = 6 per group). Group II was orally (p.o) administered sildenafil citrate (standard) at a dosage of 5 mg/kg suspended in 0.5% carboxymethylcellulose (CMC), whereas Group I was given normal saline. For four weeks in a row, three graded doses of 100, 200, and 400 mg/kg HSFE was given to Groups III, IV, and V animals (p.o.), respectively. Sexual behaviour measures were assessed at the conclusion of the therapy period.

Preparation of Male Animals

Three to four weeks, virgin male rats were acclimated before the behavioural evaluation. After a 15-minute acclimatization time frame, each male was partnered to a single female, and the two of them were monitored for 30 minutes. Dim red light was used for the study, which was conducted in a silent environment [35,36].

Preparation of Female Animals

Progesterone (1 mg/kg, s.c.) was given to female rat to trigger the estrus phase, which took place about 4 hours after therapy. Following a one-hour acclimatization period in glass cages, male rats were added for a 30-minute monitoring time. On the first, seventh, fourteenth, twenty-first, and twenty-eighth days of therapy, sexual conduct was documented [37].

Study Protocol for Male Sexual Behaviours

Prior to examination, male rats were acclimated for an hour in a 60 × 40 × 40 cm plastic cage. Estrous females were brought into the cage 10 minutes after being confirmed to be in estrus. Sexual conduct was then assessed using the following parameters: (a) Mount latency: The time between the female's introduction and the male's first mounting attempt is known as mount latency (b) Mount frequency: The total number of mounting efforts without intromission, counted from the female's introduction to ejaculation, is known as the mount frequency (c) Intromission latency: The time between the female's introduction and the male's initial intromission is known as the intromission latency [38,39].

Testosterone Serum Preparation

After administering ketamine hydrochloride to the rats to induce anesthesia, 3–5 mL of blood was extracted into sterile, anticoagulant-free syringes. After letting the samples coagulate for five to ten minutes at room temperature, they were centrifuged for ten to fifteen minutes at 3000 rpm. For the purpose of estimating testosterone levels, the clear supernatant, or serum, was meticulously collected into new tubes [40].

Testosterone analysis

Using a colorimetric technique and the Diametra testosterone ELISA kit's manufacturer's instructions, the amount of testosterone in the serum was ascertained. With 620–630 nm serving as the reference wavelength, absorbance measurements were made at 420 nm [41].

Measurement of Nitric Oxide Level

A solution consisting of 400 ml of vanadium chloride in hydrochloric acid, 200 ml of N-(1-naphthyl) ethylenediamine dihydrochloride, and 200 ml of sulfanilamide in hydrochloric acid was used to collect and homogenize penile tissues. For one hour, the mixture was kept at 37 °C. This process depends on CCl_3 converting nitrate to nitrite. Nitrite levels, which indicate the matching nitrate content, were measured spectrophotometrically at 540 nm following incubation [42].

Determination of Phosphodiesterase-5

PDE-Glo Phosphodiesterase Assay kit was used to measure PDE activity. After homogenizing the penile tissue in RIPA lysis buffer, it was centrifuged for 15 minutes at 4 °C. Following the kit's instructions, PDE-5 activity was assessed after the clear supernatant was collected. PDE-Glo termination buffer, which contains ATP and protein kinase A, has been added to the sensor solution after the sample was incubated with cyclic guanosine monophosphate (cGMP) substrate. The luciferase-based Kinase-Glo reagent, which is inversely proportional to the amount of cGMP hydrolyzed, was used to measure the level of ATP consumed during the reaction. A Spectramax L microplate luminometer (MDS AT, Stuart, FL, USA) was used to measure luminescent signed a direct related to the amount of ATP remaining and correlates with phosphodiesterase activity [43, 44].

RESULTS:

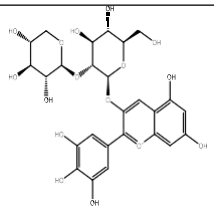
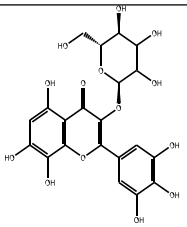
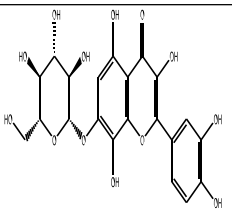
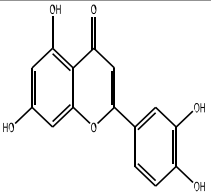
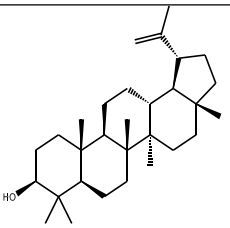
In silico study

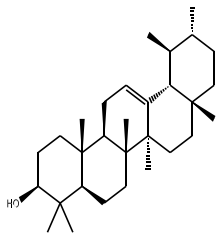
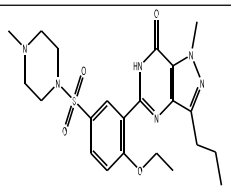
Molecular docking and ADMET

When compared to the reference inhibitor sildenafil, molecular docking tests with PDB ID: 2H42 showed that the chosen phytoconstituents had positive binding interactions. High binding affinities with numerous hydrogen bonds and hydrophobic interactions at the site of action were demonstrated by delphinidin-3-sambubioside and hibiscetin-3-glucoside among the examined drugs, indicating a stable enzyme–ligand complex. **In table 1,** all phytoconstituent's molecular docking results are presented, in **figure 1**, **(A)**: 2D interaction with amino acid residue and in **figure 1 (B),** 3D interaction images of molecules are presented. Although they had relatively lower docking scores than the glycosidic flavonoids, luteolin and gossypetin both

showed notable interactions involving residues essential for PDE-5 inhibition. The docking methodology was validated when sildenafil, as anticipated, displayed the greatest binding affinity with distinctive interactions at the active site. According to ADMET predictions, luteolin and gossypetin had good intestinal absorption and non-hepatotoxic qualities, while delphinidin-3-sambubioside and hibiscetin-3-glucoside, despite being non-mutagenic and non-carcinogenic, had limitations in oral bioavailability because of their higher polarity. ADME prediction (http://www.swissadme.ch/index.php) and Protox=3.0 TOXICITY PREDICTION (https://tox.charite.de/protox3/index.php?site=compound_input).

Table 1: Molecular docking results of Phytoconstituent's and reference with PDB ID:2H42.

Compounds name	2D Structure	Dock Score	dG Bind	2D Interaction	Hydrogen bond Distance (Å)
delphinidin-3-sambubioside		-16.719	-64.30	H-bond: ASN662, GLN775, GLN817, GLN817, LEU765 Pi-Pi Stacking: PHE820	2.64,2.33,2.01,2.25, 1.87 4.36
hibiscetin-3-glucoside		-16.970	-77.36	H-bond:ASP654, ASP764, ASP764, HIP613, ASN661, ASN662, GLN817, GLN817 Pi-Pi Stacking: PHE820	2.43,1.81,2.04,1.65, 2.01,1.92,2.08,2.33 4.11
Gossypitrin		-15.414	-68.85	H-bond:ASP654, ASP654, HIP613, ASN661, ASN661, ASN662, GLN817, GLN817 Pi-Pi Stacking: PHE786	1.86,1.64,2.31,2.06, 1.78,1.65,1.94,1.90 5.41
Luteolin		-11.552	-51.26	H-bond: ASN662, GLN817 Pi-Pi Stacking: PHE786, TYR612	2.10,2.08 5.16,5.47
Lupeol		-8.430	-83.55	No Interaction	No Distance

alpha-Amyrin		-7.896	-83.68	No Interaction	No Distance
Reference Sildenafil		-10.971	-118.52	H-bond: ASN662, GLN817, GLN817 Pi-Pi Stacking: PHE820	2.61,1.96,2.18 3.59

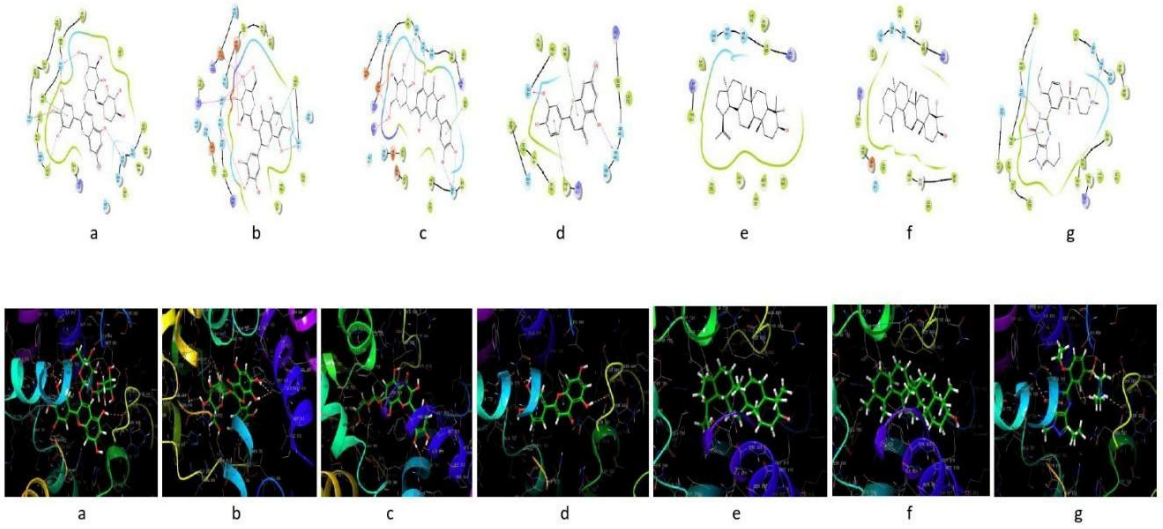


Figure 1: (A & B) 2 D & 3D images of Phytoconstituent's and reference molecules (a) delphinidin-3-sambubioside, (b) hibiscetin-3-glucoside (c) Gossypitrin (d) luteolin (e) lupeol (f) alpha-Amyrin, and (g) Sildenafil.

Molecular Dynamics (MD) Simulation

Molecular Dynamics Simulations of Phosphodiesterase-5 (PDE5) Receptor in Complex with Delphinidin-3-sambubioside, Hibiscetin-3-glucoside, Luteolin, Gossypetin, and Sildenafil.

To investigate the binding interactions between five selected ligands—Delphinidin-3-sambubioside, Hibiscetin-3-glucoside, Luteolin, Gossypetin, and Sildenafil—and the Phosphodiesterase-5 (PDE5) receptor, all-atom molecular dynamics (MD) simulations were performed for each protein-ligand complex. Simulations were conducted over a 100-nanosecond timeframe using the PDE5 crystal structure (PDB ID: 2H42). The resulting complexes included Delphinidin-3-sambubioside–PDE5, Hibiscetin-3-glucoside–PDE5, Luteolin–PDE5, Gossypetin–PDE5, and Sildenafil–PDE5. Representative structures from each simulation are presented in **Figure 2**. Trajectory analysis was used to evaluate the complexes' integrity and interaction dynamics. While mobility at the residue level was examined using root-mean-square fluctuation (RMSF), overall backbone stability was assessed using root-mean-square deviation (RMSD). In addition, hydrogen bond interactions were quantified along with their percentage occupancies over the course of the simulation, providing insights into persistent intermolecular contacts. This comprehensive, multi-parameter approach enabled a detailed characterization of the conformational behavior and binding profiles of each ligand, facilitating an evaluation of their potential efficacy as PDE5 inhibitors.

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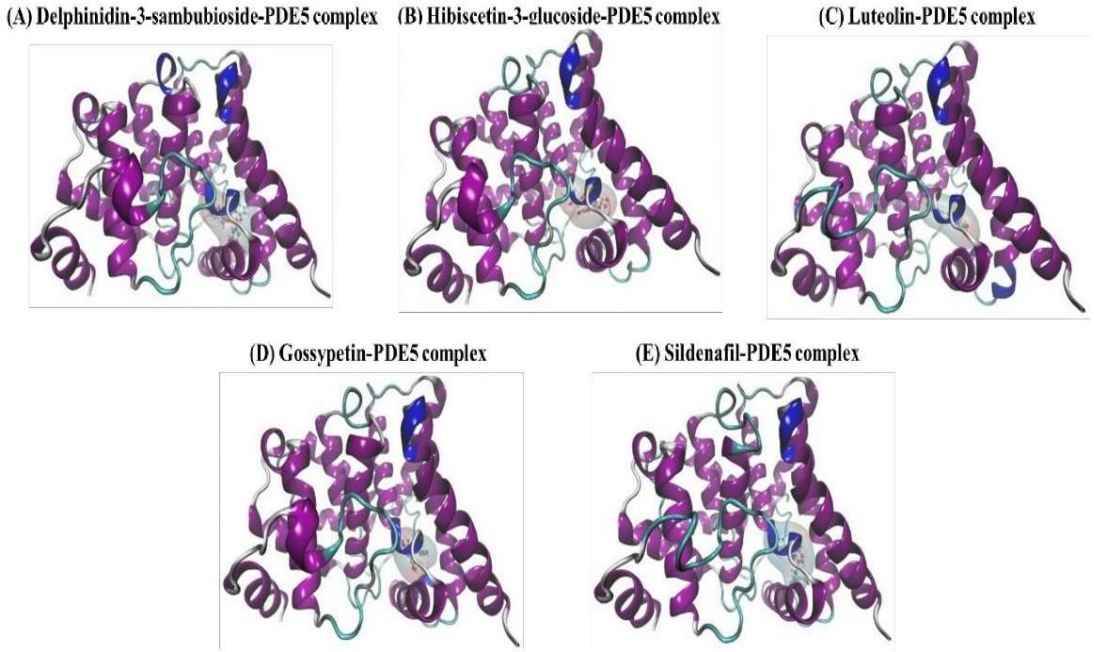

Figure 2. Structural visualization of the PDE-5-ligand complexes following molecular docking and simulation. (A) Delphinidin-3-sambubioside–PDE5, (B) Hibiscetin-3-glucoside–PDE-5, (C) Luteolin–PDE-5, (D) Gossypetin–PDE5, and (E) Sildenafil–PDE-5. The PDE-5 receptor is illustrated in cartoon representation to highlight secondary structural elements, whereas the bound ligands are shown in CPK (Corey-Pauling-Koltun) representation with a transparent molecular surface to enhance visibility of ligand positioning and interactions within the binding pocket.

RMSD analysis

Root-Mean-Square Deviation (RMSD) serves as a fundamental metric to evaluate the conformational stability of protein-ligand complexes during molecular dynamics (MD) simulations. Figure 3 (A) illustrates the RMSD trajectories for the five Phosphodiesterase-5 (PDE-5) complexes involving Delphinidin-3-sambubioside, Hibiscetin-3-glucoside, Luteolin, Gossypetin, and Sildenafil over a 100-nanosecond simulation period. All systems demonstrated average RMSD values converging near 0.3 nm, reflecting limited structural deviations and indicating overall system stability. The absence of substantial fluctuations suggests that ligand binding did not perturb the global conformation of the PDE5 protein backbone. Such stable RMSD profiles affirm the structural robustness of each complex.

Ligand RMSD analysis offers critical insights into the dynamic behaviour and binding stability of small molecules within the receptor binding site over the course of molecular dynamics (MD) simulations. As illustrated in Figure 3 (B), the Delphinidin-3-sambubioside–PDE5 and Hibiscetin-3-glucoside–PDE-5 complexes displayed highly stable ligand RMSD profiles, with average deviations of approximately 0.15 nm and 0.2 nm, respectively. These minimal fluctuations indicate sustained and stable interactions between the ligands and the PDE5 binding pocket throughout the 100 ns simulation. In comparison, the remaining complexes—Luteolin–PDE-5, Gossypetin–PDE-5, and Sildenafil–PDE-5—also exhibited stable ligand RMSD values, though with slightly greater fluctuations, all remaining within 0.2 nm. These modest variations are indicative of flexible yet stable binding behaviour, suggesting that the ligands remain well-accommodated within the active site despite minor conformational adjustments during the simulation. Collectively, these results highlight Delphinidin-3-sambubioside and Hibiscetin-3-glucoside as the most conformationally stable ligands in complex with PDE-5. However, the comparable stability observed for Luteolin, Gossypetin, and Sildenafil underscores their continued relevance as potential PDE5 inhibitors.

RMSF analysis

One important method for assessing the adaptability of individual amino acid residues in a protein during molecular dynamics (MD) simulations is Root-Mean-Square Fluctuation (RMSF) analysis. This method provides critical insights into localized

conformational dynamics that may influence ligand binding and protein function. Figure 3 (C) illustrates the comparative RMSF profiles of the five PDE-5-ligand complexes: Delphinidin-3-sambubioside–PDE-5, Hibiscetin-3-glucoside–PDE-5, Luteolin–PDE-5, Gossypetin–PDE-5, and Sildenafil–PDE5. The fluctuations are expressed in nanometers and plotted against residue indices of the PDE5 protein. Across all complexes, the majority of residues exhibited RMSF values below 0.2 nm, indicating overall structural rigidity and minimal deviation from the native conformation. Regions with slightly elevated fluctuations likely correspond to flexible loop segments or residues within or near the active site, which are typically involved in ligand accommodation and dynamic enzymatic processes. Importantly, no significant destabilization was observed upon ligand binding, suggesting that these compounds do not adversely affect the structural coherence of the PDE5 receptor. These observations support the hypothesis that the studied ligands are compatible with the PDE5 binding environment and may possess favourable dynamic properties for therapeutic targeting.

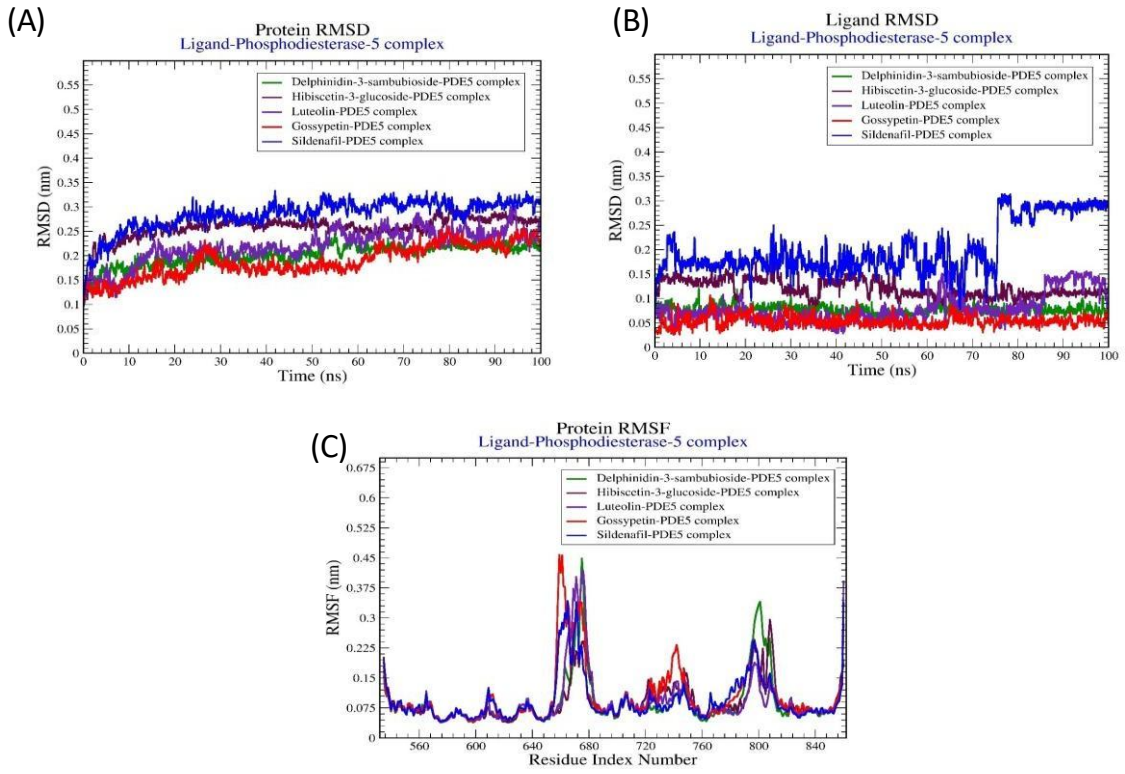

Figure 3. (A): RMSD profiles of PDE5-ligand complexes over a 100 ns molecular dynamics simulation. The plots depict the temporal evolution of protein backbone RMSD (in nm) for each complex. **(B):** Ligand RMSD trajectories for PDE-5-ligand complexes over a 100 ns molecular dynamics simulation. The plots depict the Root-Mean-Square Deviation (RMSD) of each ligand with respect to its initial binding conformation. **(C):** RMSF profiles of PDE5 residues in complex with selected ligands over a 100 ns molecular dynamics simulation. The plots display Root-Mean-Square Fluctuation (RMSF) values (in nm) plotted against the residue index of the PDE5 protein for each complex.

H-bond interaction

Hydrogen bonding (H-bond) analysis was conducted to examine the strength and temporal stability of interactions between each ligand and the Phosphodiesterase-5 (PDE-5) receptor throughout the 100 ns molecular dynamics (MD) simulations. All five ligand-PDE-5 complexes exhibited hydrogen bond formation during the simulation, with most ligands forming up to three H-bonds on average. Among the complexes, Delphinidin-3-sambubioside–PDE5 and Hibiscetin-3-glucoside–PDE5 showed notably stronger and more persistent interactions, maintaining up to six hydrogen bonds consistently across the entire simulation trajectory (Figure 4). This high level of H-bond occupancy reflects a stable and tightly bound interaction within the active site of PDE-5. In contrast, the Luteolin–PDE5, Gossypetin–PDE5, and Sildenafil–PDE-5 complexes displayed a biphasic interaction pattern. During the initial 60 ns, these ligands formed a limited number of hydrogen bonds—

approximately four for Luteolin, two for Gossypetin, and four for Sildenafil—indicating a weaker initial interaction. However, in the final 40 ns of the simulation, an increase in H-bond formation was observed, with Luteolin and Sildenafil reaching up to five and four hydrogen bonds, respectively, while Gossypetin maintained two. This late-stage increase suggests a dynamic rearrangement of ligand-receptor contacts, possibly due to conformational adaptations within the PDE-5 binding pocket. These findings highlight both the variability and adaptability of hydrogen bonding patterns among the studied ligands. Delphinidin-3-sambubioside and Hibiscetin-3-glucoside, in particular, demonstrated more stable and sustained interactions, reinforcing their potential as high-affinity PDE-5 inhibitors. Overall, the results underscore the critical role of hydrogen bonding in ensuring the stability and efficacy of ligand binding to the PDE-5 receptor.

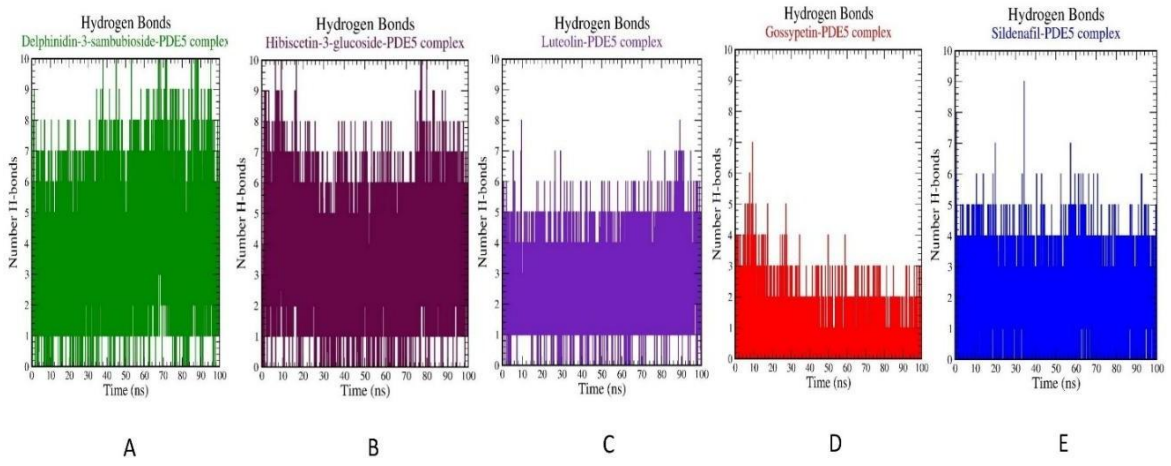

Figure 4. Time-dependent hydrogen bond analysis of PDE5-ligand complexes over a 100 ns molecular dynamics simulation. The plots depict the number of hydrogen bond (H-bond) contacts formed between each ligand and the Phosphodiesterase-5 (PDE-5) receptor (PDB ID: 2H42): (A) Delphinidin-3-sambubioside–PDE-5, (B) Hibiscetin-3-glucoside–PDE-5, (C) Luteolin–PDE5, (D) Gossypetin–PDE5, and (E) Sildenafil–PDE5. These graphs highlight the temporal variation in H-bond occupancy, providing insights into the stability and strength of ligand-receptor interactions throughout the simulation.

Hydrogen bond occupancy analysis revealed diverse and ligand-specific interaction patterns between the five PDE5-ligand complexes and various amino acid residues of the Phosphodiesterase-5 (PDE-5) receptor (Figure 5). In the Delphinidin-3-sambubioside–PDE5 complex, the most frequent interaction was observed with GLN817, exhibiting a cumulative occupancy of 149.47%. Additional notable interactions were recorded with PHE820 (65.37%), ASN662 (36.21%), TYR612 (24.83%), and MET816 (22.83%). Interestingly, a significant portion of occupancy (78.42%) was attributed to residues classified as "OTHER," suggesting a broader and potentially non-specific interaction profile. For, the Hibiscetin-3-glucoside–PDE-5 complex, the highest occupancy was observed with ASP764 at 158.70%, accompanied by relevant contributions from GLN817 (53.25%), MET816 (24.34%), PHE820 (19.44%), and HIS613 (17.37%). Similar to Delphinidin-3-sambubioside, this complex also showed a high proportion of interactions (81.47%) under the "OTHER" category, implying a degree of conformational flexibility in its binding mode. In the Luteolin–PDE5 complex, ASP764 emerged as the primary interaction site with an exceptionally high occupancy of 233.12%, followed by GLN817 (15.03%), THR723 (8.10%), LEU725 (5.22%), and LEU804 (3.10%). For the Gossypetin–PDE5 complex, comparatively weaker interactions were observed, with ILE768 (5.68%), VAL782 (5.90%), PHE786 (6.33%), ILE813 (6.58%), and GLN817 (7.04%) contributing to the total occupancy. Notably, "OTHER" residues accounted for 27.70%, indicating moderate binding variability. In contrast, the Sildenafil–PDE5 complex demonstrated a more specific interaction profile, dominated by GLN817 (131.20%), followed by MET816 (14.36%), PHE820 (12.47%), LEU804 (6.01%), and ILE768 (3.47%). The "OTHER" category contributed only 13.43%, highlighting a more focused binding interaction compared to the other ligands. Over all, Delphinidin-3-sambubioside and Hibiscetin-3-glucoside exhibited extensive and persistent hydrogen bond interactions across key PDE-5 residues, suggesting their strong binding affinity and potential as promising candidates for PDE5 inhibition. These findings support the further investigation of these two phytochemicals as potential therapeutic agents targeting erectile dysfunction via PDE-5 modulation.

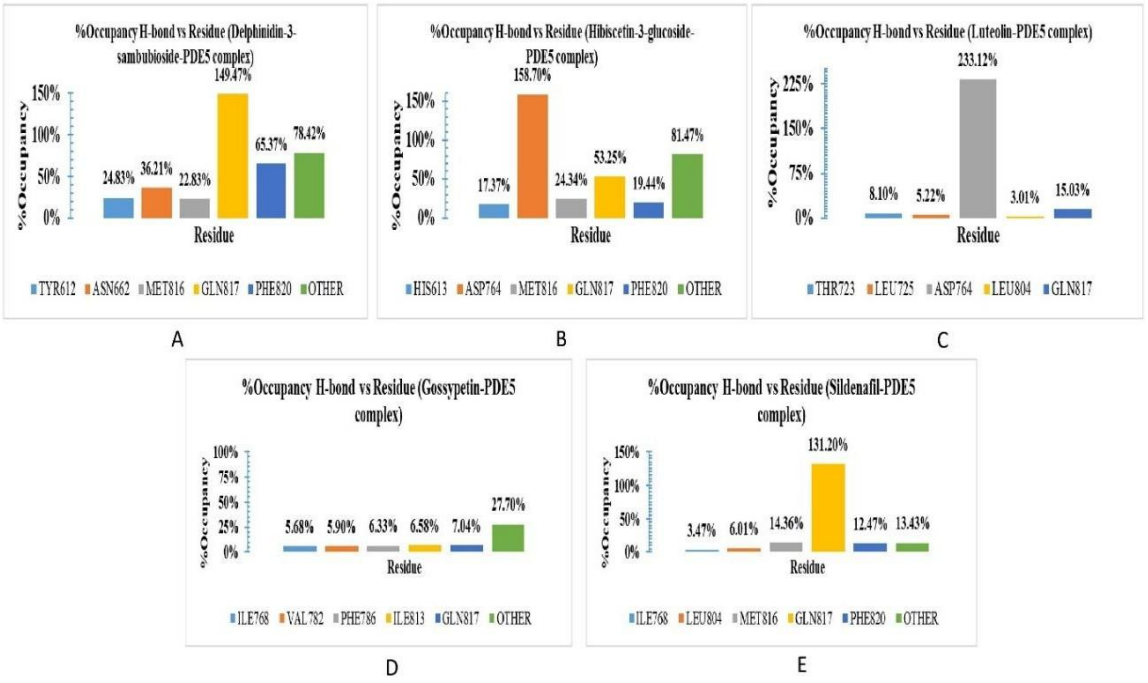

Figure 5. Histogram illustrating the percentage occupancy of hydrogen bond interactions between PDE5 (PDB ID: 2H42) and each ligand over a 100 ns molecular dynamics simulation. The plots represent residue-wise H-bond occupancies for the following complexes: (A) Delphinidin-3-sambubioside–PDE5, (B) Hibiscetin-3-glucoside–PDE5, (C) Luteolin–PDE5, (D) Gossypetin–PDE5, and (E) Sildenafil–PDE5. The data highlight key residues contributing to stable protein-ligand interactions and indicate differences in binding specificity and strength among the studied compounds.

Effect of HSFE on the Mount frequency, Mout Latency and Intermission Latency

Oral administration of HSFE doses of 100, 200, and 400 mg/kg produced a dose-dependent improvement in male sexual behaviour parameters. Mount frequency (MF) (Figure 6 A) was significantly increased in all treated groups compared to the normal control, with the highest activity observed at 400 mg/kg, which was comparable to the sildenafil-treated group. Conversely, mount latency (ML) (Figure 6 B), and intromission latency (IL) (Figure 6 C) were significantly reduced in extract-treated animals, indicating enhanced sexual motivation and improved copulatory performance. At the lowest dose (100 mg/kg), the effect was moderate but still statistically different from the control group. The 200 mg/kg dose showed a substantial improvement, while 400 mg/kg exhibited near-similar efficacy to sildenafil, confirming the potential pro-sexual activity of the extract.

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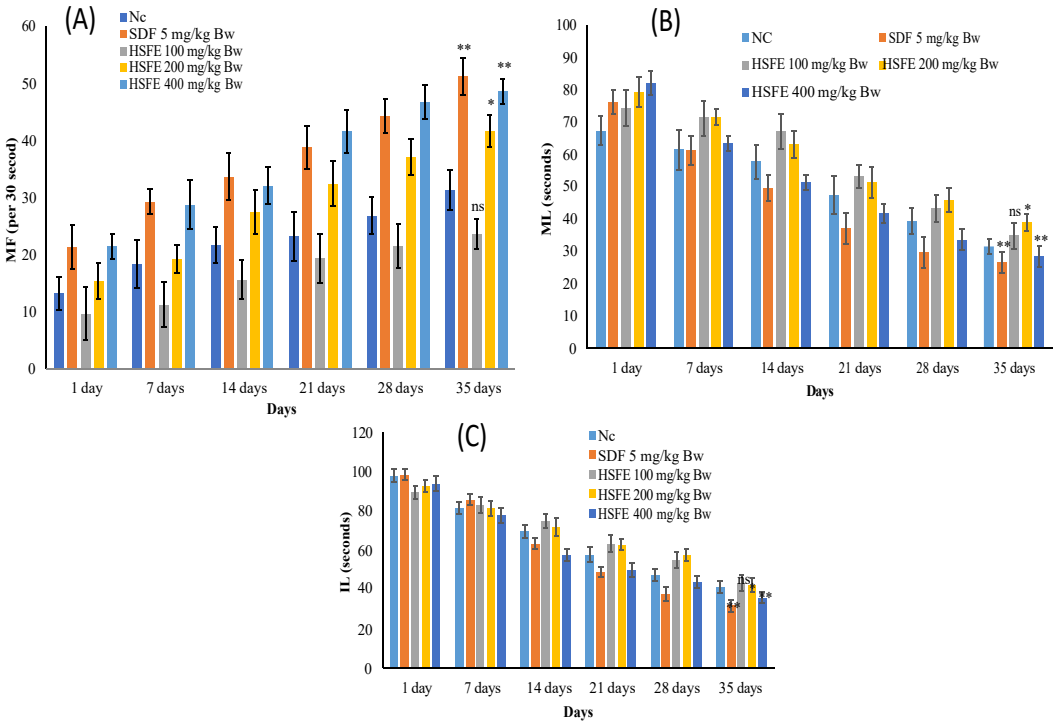

Figure 6 (A, B & C): HSFE's effect on mount frequency, mount latency and Intermission Latency (n = 6). The data are means ±SEM; * $p < 0.05$, ** $p < 0.01$; ns $p > 0.05$ (followed by Student Dunnett test).

2.8 Body Weight: In experimental rats, giving them of HSFE at doses of 100, 200, and 400 mg/kg showed a dose-dependent impact on body weight. The body weight of the sildenafil-treated group increased 41.03% during the study period, while that of the normal control group increased 29.77%. Body weight significantly improved in dose-dependent manners in animals treated with HSFE, especially at 100, 200, and 400 mg/kg (31.29%), (36.94%), and (39.2%), (Figure 7) indicating improved nutritional utilization and general health state. There was no statistically significant effect observed in the 100 mg/kg group. Higher dosages of *H. sabdariffa* extract may have a beneficial effect on metabolic activity and aid in weight regulation, according to these findings.

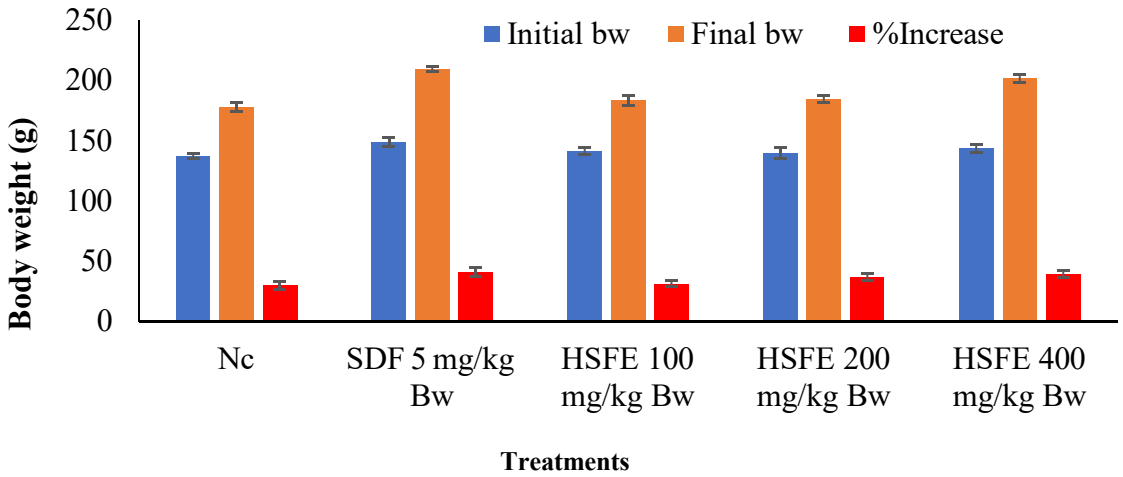

Figure 7: HSFE's effect on body weight (n = 6). The values are means ±SEM.

Testosterone levels

Serum testosterone levels increased in a dose-dependent manner when HSFE was administered at doses of 100, 200, and 400 mg/kg in comparison to the normal control group. The testosterone concentrations in the 200 mg/kg and 400 mg/kg groups increased significantly, although the low dose (100 mg/kg) exhibited a slight increase. The highest effect among the tested doses was 400 mg/kg, which was close to values similar to those of the sildenafil-treated group. According to statistical analysis, the sildenafil group maintained the largest rise ($p < 0.001$ vs. control), while the 200 mg/kg and 400 mg/kg doses considerably ($p < 0.05$ and $p < 0.01$, respectively) raised blood testosterone in comparison to the control. (Figure 8). These results imply that extract from *H. sabdariffa* increases androgenic activity in a dose-dependent way.

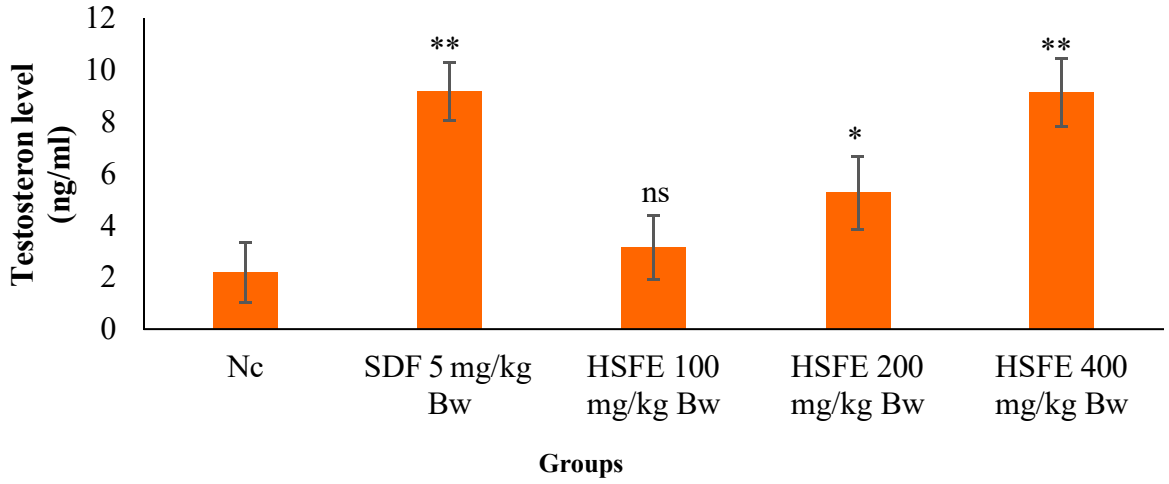

Figure 8: HSFE's on testosterone level (n = 6). The data are means ±SEM; * $p < 0.05$, ** $p < 0.01$; ns $p > 0.05$ (followed by Student Dunnett test by GraphPad Prism).

Measurement of Nitric Oxide Level

All treatment groups experienced a dose-dependent increase in nitric oxide levels following the administration of HSFE. When compared to the normal control, animals treated with 100 mg/kg showed a mild but significant increase ($p < 0.05$). In contrast, animals treated with 200 mg/kg and 400 mg/kg showed a more noticeable rise, with the largest response occurring at 400 mg/kg ($p < 0.01$ vs. control). (Figure 9). The highest dose had an effect similar to that of the group treated with sildenafil, suggesting that HSFE may improve the production of endothelial nitric oxide. These results imply that the extract might promote vascular relaxation by modulating the nitric oxide pathway, which would be the mechanism by which it exerts its pharmacological action.

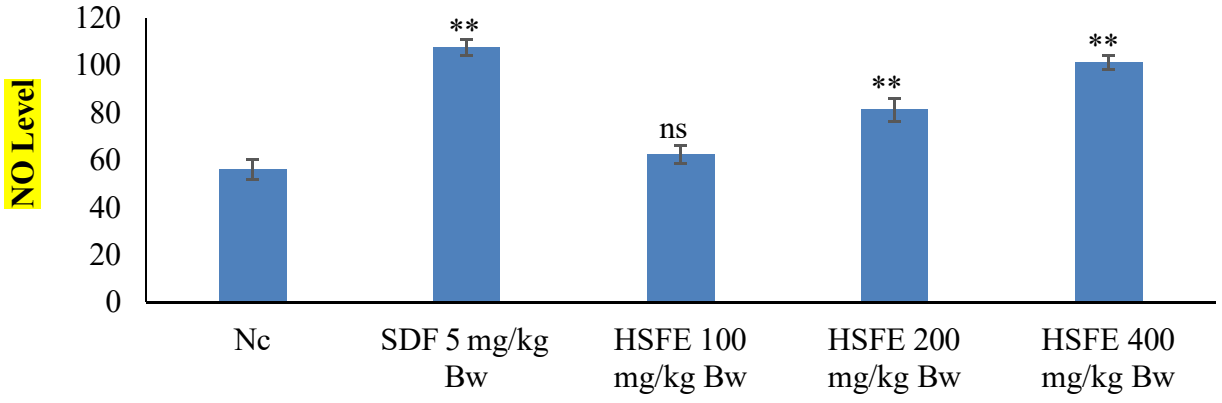

Figure 9: HSFE's effect on NO level (n = 6). The data are means ± SEM; * $p < 0.05$, ** $p < 0.01$; ns $p > 0.05$ (followed by Student Dunnett test GraphPad Prism).

Determination of Phosphodiesterase-5

Phosphodiesterase-5 (PDE-5) activity was significantly and dose-dependently inhibited when *Hibiscus sabdariffa* flower extract was administered at doses of 100, 200, and 400 mg/kg in comparison to the normal control group. At 400 mg/kg, the greatest effect was seen, with inhibition that was similar to but somewhat less pronounced than that of the sildenafil-treated group. PDE-5 activity was moderately but significantly reduced by the 200 mg/kg dose, whereas the 100 mg/kg dose had a minor effect. (Figure 10). As a positive control, the sildenafil group, on the other hand, displayed the greatest inhibition. These results confirm the traditional use of *H. sabdariffa* to enhance erectile function by indicating that it contains phytoconstituents with possible PDE-5 inhibitory action.

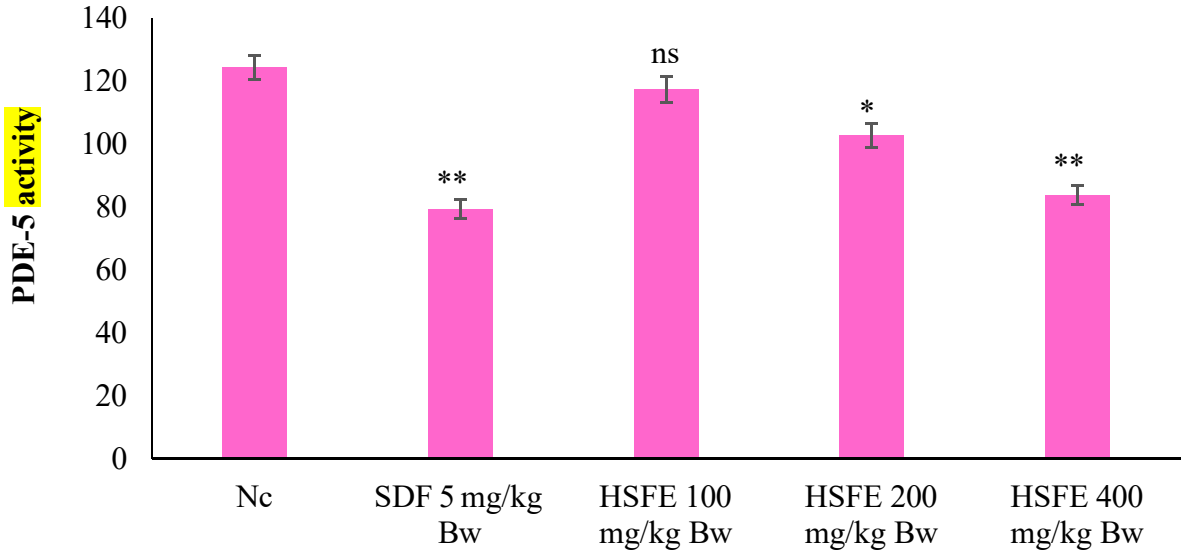

Figure 10. HSFE's effect on phosphodiesterase level (n = 6). The data are means ±SEM; * p <0.05, ** p < 0.01; ns p > 0.05

DISCUSSION:

The current study offers thorough proof that HSFE has notable pro-sexual benefits, which can be attributed to its several modes of action, including nitric oxide modulation, testosterone regulation, and PDE-5 inhibition. Phytoconstituents such gossypitrin, luteolin, hibiscetin-3-glucoside, and delphinidin-3-sambubioside showed substantial binding affinities for PDE-5, sometimes outperforming the conventional reference medication sildenafil, according to the in-silico studies. These results are in line with past studies showing how flavonoids and anthocyanins might alter PDE enzymes and endothelial function to enhance erectile function [45,46]. The stability of ligand–enzyme complexes has been confirmed by molecular dynamics simulations, hence confirming the natural chemicals' pharmacological significance.

With dose-dependent increases in sexual behaviour measures, such as mount frequency and decreases in mount and intromission latency, the in vivo data supported the computational expectations. These results are consistent with other experiments where polyphenols derived from plants enhanced penile hemodynamics and smooth muscle relaxation to increase sexual activity [47-48]. Crucially, the extract increased nitric oxide concentration and serum testosterone levels, which are essential for controlling libido, erectile function, and penile vasodilation [49]. Compared to single-target pharmacotherapies, H. sabdariffa may have broader benefits because to its simultaneous regulation of vascular and hormonal pathways.

It appears that flavonoids like luteolin, that have been shown to have vasodilatory and antioxidant properties [50,51], may possibly contribute to *H. sabdariffa's* extract PDE-5 inhibitory action in addition to anthocyanins like delphinidin derivatives. These phytochemicals may work in concert to enhance nitric oxide bioavailability, endothelial function, and oxidative stress suppression—all of which are frequently weakened in erectile dysfunction. Furthermore, the extract's ability to work across a

variety of routes suggests that there may be a lower chance of resistance development, which is a worry related to long-term use of PDE-5 inhibitors [52-53].

In order to lower the risk of erectile dysfunction (ED), lifestyle changes, weight control, and ideal lipid management are crucial. High-density lipoprotein (HDL) and triglyceride (TG) levels are helpful markers of visceral adipose tissue dysfunction and abdominal obesity [54]. Excess visceral fat contributes to endothelial dysfunction and ED by stimulating the release of pro-inflammatory mediators such as adipokines, interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α). Male sexual function is further hampered by increased aromatase activity in visceral adipose tissue, which increases the conversion of testosterone to oestradiol [55,56].

When combined, these findings highlight the potential of HSFE as a phytotherapeutic option for treating erectile dysfunction. A strong basis for upcoming translational research is provided by the convergence of behavioural results, biochemical validation, and computational predictions. To standardize bioactive fractions, create pharmacokinetic profiles, and assess safety in clinical settings, more investigation is necessary. The development of natural, affordable supplements or substitutes for the existing medication for male sexual dysfunction may be made easier by such research.

LIMITATION:

This study's dependence on a single animal model (rats), which might not accurately reflect human sexual physiology and erectile dysfunction pathophysiology, is a significant disadvantage. Long-term safety and efficacy evaluation was hampered by the relatively small sample size and treatment duration. Strong PDE-5 binding is shown by molecular docking and MD simulations, however these in silico results might not immediately transfer into clinical results. In-depth pharmacokinetic and bioavailability information for the identified phytoconstituents—which are crucial for therapeutic application—is also absent from the study. Additionally, the extract's standardization, drug–herb interactions, and possible toxicity were not thoroughly discussed. Lastly, the lack of clinical validation restricts the direct application of these results to human participants, requiring additional carefully planned preclinical and clinical studies.

CONCLUSION:

The current study shows that HSFE has strong pro-sexual effects via a variety of multimodal processes, such as testosterone modulation, nitric oxide increase, and PDE-5 inhibition. The significant binding affinity of important phytoconstituents to PDE-5 has been demonstrated by computational docking and MD simulations, and in vivo results verified dose-dependent enhancements in biochemical markers and sexual behaviour. All of these findings point to the potential of H. sabdariffa flower extract as a natural treatment option for erectile dysfunction and call for more preclinical and clinical research.

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