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Male Infertility Intervention: An In-Silico Analysis of Phytocompounds from Rauwolfia Vomitoria

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Abstract; The increasing rate of infertility in men worldwide raises concerns about the side effects of available drugs. This calls for continuous efforts towards the discovery of natural agents targeting important pathways implicated in male infertility. An ethnobotanical survey of Rauwolfia vomitoria Afzel, a plant family, was used to identify potential inhibitors of cyclooxygenase-2 (COX-2) and phosphodiesterase type 5 (PDE5) from bioactive compounds. 106 compounds were screened using molecular docking analysis followed by ADMET (absorption, distribution, metabolism, excretion and toxicity) study. Heneicosanol from Rauwolfia vomitoria had better physicochemical and ADMET properties than LUR for the COX-2 receptor, making it a potential anti-inflammatory compound. The compounds interacted with various proteins, indicating they possess the binding affinities and molecular interactions required for inhibitors of COX-2 and PDE5, potentially reducing inflammation and improving erectile function.The compounds also have comparative Molecular mechanics with generalised Born and surface area solvation (MMGBSA) and relative ADMET analysis with the co-factor. Hence, these compounds could be said to possess the binding affinities and molecular interactions required as inhibitors of cyclooxygenase-2 (COX-2) and Phosphodiesterase type 5 (PDE5) which could help in to reduce inflammation leading to infertility in male and improve erectile function

keywords: Male infertility, Rauwolfia vomitoria, molecular docking, anti-inflammatory, erectile dysfunction

Introduction

Male infertility is any health issue in a man that lowers the chances of his female partner getting pregnant. Male infertility is emerging as an important cause of infertility worldwide accounts for 30% of infertility cases and its prevalence in the

general population approximately ranges between 9 and 15% (Caroppo and Colpi, 2023). There are evidences to show that sperm counts have been declining over the last 50 years, with a consequent increase in male infertility (Olayemi, 2010).

Male infertility generally caused by a variety of non-exclusive causes and contributory factors, including congenital and genetic factors, anatomical dysfunctions, hormonal disorders, ejaculatory dysfunctions, genital tract infections, immunological abnormalities, chronic diseases, cancer and related treatments, gonadotoxin exposure, insufficient lifestyle and hormonal imbalance (Azenabor et al., 2015) and inflammation (Roy Choudhury & Knapp, 2001). Environmental factors, such as exposure to harmful substances and stress associated with unhealthy lifestyles, have also been linked to health risks in humans (Chao et al., 2023).

There are numerous factors causing inflammation of the male reproductive tract. Among these are ejaculatory duct obstruction, Epididymitis, Testicular torsion, varicocele, urogenital obstruction and orchitis (inflammation of one or both testes) (Azenabor et al., 2015). The association between inflammation and infertility in male is an important issue in modern medicine. During inflammatory processes, reduction in semen quality is as a result of several factors from obstruction of sperm transport, dysregulation of spermatogenesis and impairment of accessory gland functions. In infertile males with reproductive problems associated with inflammation urogenital tract not caused by infection, NSAIDS (non-steroid anti-inflammatory drugs) are usually recommended. This has shown to inhibit the production of prostaglandins which are synthesized from arachidonic acid by the action of cyclooxygenase (COX) enzymes (Gambera et al., 2007). The principal mechanism of NSAIDS is COX inhibition. COX exists in two isoforms: COX-1 and COX-2. While COX-1 is constitutively expressed and is involved in PGE synthesis in many tissues and organs, such as the testis, COX-2 is induced by endotoxins, growth factors, and inflammatory cytokines.COXCyclooxygenase enzymes (COX-1 and COX-2) catalyze the conversion of arachidonic acid to prostaglandin G2 (Dawood et al., 2015). Numerous side effects are linked with prolong use of NSAIDs which have resulted in the production of many COX-2 selective inhibitors with an enhanced gastrointestinal wellbeing (Boschetti, 2021). However, many of the NSAIDs have been reported to cardiovascular, myocardial infarction renal side effects (Dawood et al., 2015).

Another major cause of male infertility is erectile dysfunction (ED). ED is the failure to achieve or maintain a rigid penileerection suitable for satisfactory sexual intercourse(Muneer et al., 2014). Erectile dysfunction may have organic or psychogenic causes. Organic cause may include neurovascular, hormonal and anatomical factor leading to erectile dysfunction. Psychogenic erectile dysfunction may be attributed to psychological disorders, such as schizophrenia or depression which is further exacerbated by drugs prescribed to treat these diseases, performance anxiety or relationship stress (Montorsi & Salonia, 2004). Lifestyle is

also a common cause of ED. Presently, quite a few drugs are existing for treating erectile dysfunction. First on the list of options is oral phosphodiesterase (PDE) inhibitors, others may include transurethral prostaglandins and Intra-cavernosal injections (Muneer et al., 2014). A phosphodiesterasetype 5 inhibitor (PDE5 inhibitor) is avasodilating drug that works by blocking the degradative action of cyclic guanosine monophosphate (cGMP) to GMP in the smooth muscle cells lining the blood vessels supplying various tissues. Phosphodiesterase 5 (PDE5) inhibitors are a type of drug that can affect blood flow and how cells communicate in the body (Sissons, 2022).The most common side effects seen with sildenafil include headache, flushing, dyspepsia, and rhinitis. The adverse effects with tadalafil and vardenafil are similar to sildenafil, although tadalafil is associated with a higher incidence of back pain and myalgia(Brock et al., 2002).It is therefore imperative to continuously search for sources of COX-2 inhibitors and PDE5 inhibitors from medicinal sources to combat the various side effects.

Various medicinal plants in Africa have been reported to elicit varying benefit on the male reproductive system and one of the is Rauwolfia vomitoria Afzel(Owolabi et al., 2024, Toyin et al., 2014). Rauwolfia vomitoria Afzel, belong to plant family Apocynaceae which is native to various regions in the Africa continent but mainly found in Nigeria, Cameroon, Ghana and other West African countries. The plant is a small to medium-sized evergreen tree, with dark green, glossy leaves that are oblong or elliptical in shape. The bark is originally a smooth and grayish-brown but matures to a dark brown with a rougher texture (Olubunmi, 2022). The common names are African serpent wood, African snakeroot, Poison Devil Pepper, Smooth Brown Bark in English while the local names Asofeyeje, ira-igbo(Yoruba), Aduwawa (Edo), Wadda, Kukumaka(Hausa), Mmoneba, Utoenyin (Efik), Akata (Bini) (Ehiagbonare, 2007).Rauvolfia vomitoria has been documented for its numerous therapeutic effects and extensively use for countless diseases especially as an antimalarial, in the lowering of blood pressure; possesses analgesic, haematinic and antipyretic properties (Olatokunbohet al., 2009). Also, useful in the treatment of diarrhea and male infertility (Toyin et al., 2014).

However, there's scarcity of information on the actual bioactive components accountable for the mechanism of action of most of the medicinal claims. Hence, this study aims to use in silico analysis to identify specific phyto-components likely responsible for the improvement of male infertility on COX-2 inhibitor and PDE5 inhibitor protein targets representing different mechanism of action.

Materials and Methods

Bioactive data Generation

In this study 106 phytocompounds previously identified and characterized from R. vomitoria were retrieved from PubChem (pubchem.ncbi.nlm.nih.gov) in two-

dimensional (2D) structure data files (SDF). All phytochemicals used were on Table 1 in the supplementary attachments. On the other hand, two protein targets implicated in male infertility (a) Cyclooxygenase-2 (COX-2) Inhibitor and (b) PDE5 Inhibitors were obtained from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (www.rcsb.org) with PDB ID 4RRW and 1XOZ respectively. 1XOZ has a resolution of 1.37 Å and 2.57 Å for 4RRW

Computational Analysis

The computational analysis performed in this study was done on Schrodinger suite Maestro 2018–1, these includes Ligprep, Protein preparation, Grid generation and Glide XP docking.

Ligand Preparation, Preprocessing and preparation of protein target structures

All the 106 phytochemicals previously downloaded were imported into Maestro, these were prepared by the ligprep tool on the software. Later phytochemicals were optimized by addition of hydrogen atoms, ionizing at pH (7.2 ± 0.2), and removing salt. Epik with OPLS3 force field for protonation, stereoisomerization, tautomers generation and to achieve biological conformers (Brooks et al., 2008).

Protein X-ray crystal structures of Cyclooxygenase-2 (COX-2) Inhibitor and PDE5 Inhibitors obtained from the Protein Data Bank were loaded into Maestro The protein preparation wizard tool on Maestro Suite was then used to prepare the targets by removing water molecules, assigning bond orders, adding hydrogen, creating disulfide bonds and generating HET states. The H-bond optimization was also done to refine the before minimization using OPLS3e force field was done (Esther et al., 2017)

Receptor Grid Generation

The interface for docking was generated with the receptor grid generation tool in maestro. This created a cubic box when the co-crystallized ligand was selected to map out the binding site on the receptor where the ligands will be docked.

Molecular docking analysis

The Ligand Docking tool on Maestro's Schröndinger suite was used to perform molecular docking with the prepared phytochemicals being docked in the active site (the co-crystallized ligands were first extracted although re-docked into the site to authenticate the docking protocol while it served as the standard) specified by the grid on the minimized protein while the ligands are kept flexible. Glide Extra precision (XP) tool is used for the justification of suitable ligand molecule to the active site of specific target (Omotuyi et al., 2023).

Estimation of ligand binding free energy using Prime-MM-GBSA

The docked protein-ligand complex binding free energy was estimated using Prime MM-GBSA (Molecular Mechanics/Generalized Born Surface Area) module in Schrodinger Suite (Esther et al., 2017)

Hits' Drug-likeness: pharmacokinetic and pharmacochemical analysis

The ADMET (absorption, distribution, metabolism, excretion and toxicity) predictions for the top docking hits were subjected to online software Admetlab 2.0. Various parameters related to physicochemical descriptors, drug likeness and pharmacokinetic were predicted(Jahangeer et al., 2023)

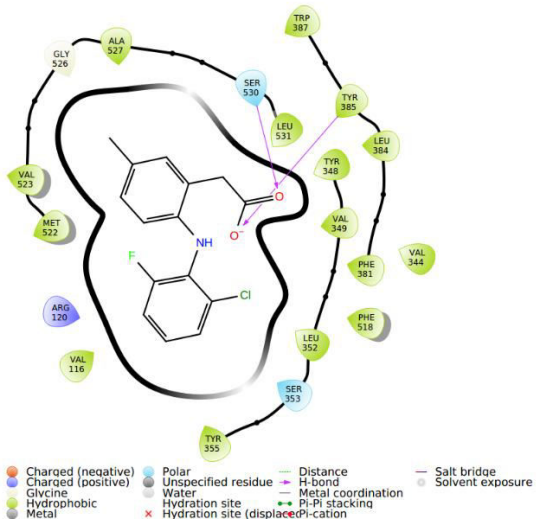
RESULTS

Table 1 represent the results of ligand docking analysis of protein target of COX-2 (4RRW) with phytochemicals from Rauwolfia vomitoria. From the table, the co-crystallized ligand (LUR-Lumiracoxib) had the best docking score of -10.981 kcal/mol, glide energy of -44.409 kcal/mol, glide Emodel of -67.667 and MMGBSA of -52.82 kcal/mol, However, phytocompound Heneicosanol, has MMGBSA score (-51.01) close to that of LUR and the docking score of -9.759.

Table 1: Extra Precision Glide Docking of Cyclooxygenase-2 (COX-2) Inhibitor

S.No	Compound ID	PUBCHEM ID	Glide Docking Score (kcal/mol)	Glide Energy (kcal/mol)	Glide Emodel	MMGBSA dG Bind (kcal/mol)
1	LUR _Lumiracoxib	151166	-10.981	-44.409	-67.667	-52.82
2	Heneicosanol	85014	-9.759	-37.619	-51.228	-51.01
3	1_(1_Phenyl_2_ phenylsulfonylaminoethyl)aziridine	583292	-9.445	-39.272	-55.869	-28.23
4	Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1	572764	-9.157	-14.754	10000	3.12
5	Eicosanoic Acid	10467	-8.955	-33.722	-17.042	-20.84

Figure 1 is the 2D graphical representation of protein- ligand interaction of target 4RRW and selected phytochemicals of Rauwolfia vomitoria. These were further detailed in Table 2. LUR had about 14 hydrophobic interactions and 2 hydrogen bond interactions while Heneicosanol had about 18 hydrophobic interactions with 2 hydrogen bonds. However, 1(1_Phenyl_2_ phenylsulfonylaminoethyl) aziridine in addition to hydrophobic and hydrogen bond had Pi-Pi stacking.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LEU 531
LEU 384
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VAL 349
MET 522
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PHE 518
LEU 352
VAL 116
SER 353
TYR 355
NH
O
F
Cl
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
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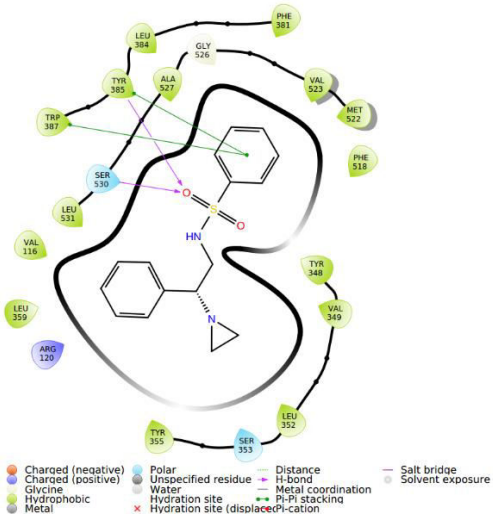
Hydration site (displaced
Distance
H-bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

A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VAL 349
ARG 120
LEU 352
TYR 355
SER 353
HN
N
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Hydrophobic
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Polar
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Pi-cation
Salt bridge
Solvent exposure

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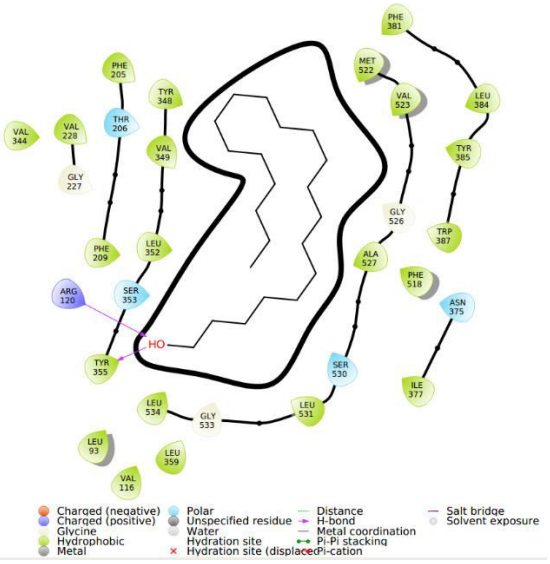
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GLY 533
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Salt bridge
Solvent exposure

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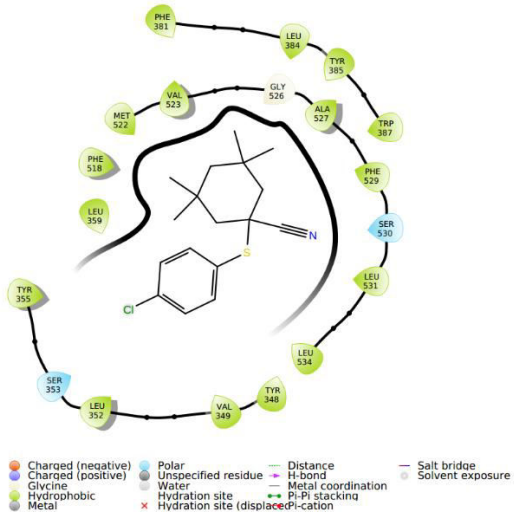
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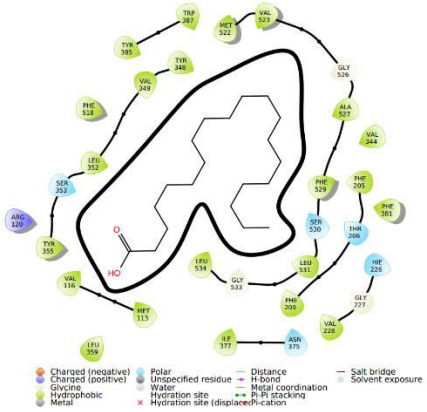
Solvent exposure

D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SER 353
SER 530
PHE 381
ARG 120
THR 206
O
TYR 355
HO
LEU 534
LEU 531
HIE 226
GLY 533
VAL 116
PHE 208
GLY 227
MET 113
HIE 377
ASN 375
VAL 228
LEU 359
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced
Distance
H-bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

E

Figure 1: Two-dimensional (2D) molecular interactions of amino acid residues of Cyclooxygenase-2 (COX-2) Inhibitor (4RRW) with hit compounds of Rauwolfia vomitoria (A) Lumiracoxib (B) 1(1_Phenyl_2_phenylsulfonylaminoethyl) aziridine (C) Heneicosanol (D) Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1 (E) Eicosanoic Acid

TABLE 2: Ligand interaction of Cyclooxygenase-2 (COX-2) Inhibitor with selected Phytochemicals from *Rauwolfia vomitoria*

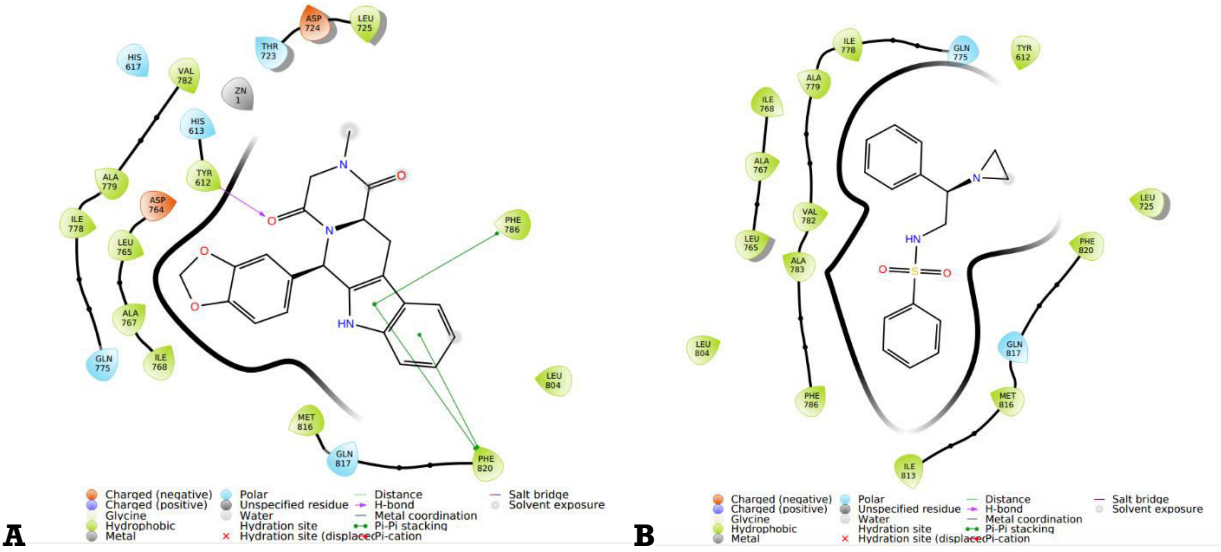
S.No	Compound ID	Interacting amino acids
1	LUR _Lumiracoxib	Hydrophobic-VAL 344, TYR 348, VAL 349, LEU 352, TYR 355, PHE 381, LEU 384, TYR 385, TRP 387, MET 522, VAL 523, GLY 526, ALA 527, LEU 531 H-Bond- TYR 385, SER 530
2	Heneicosanol	Hydrophobic- PHE 205, PHE 209, VAL 228, VAL 344, TYR 348, VAL 349, LEU 352, TYR 355,ILE 377, LEU 384, TYR 385, TRP 387, PHE 518,MET 522, VAL 523, ALA 527, LEU 531, LEU 534 H-Bond- ARG 120, TYR355
3	1(1_Phenyl_2_ phenylsulfonylaminoethyl) aziridine	Hydrophobic- TYR 348, VAL 349, LEU 352, TYR 355, PHE 381, LEU 384, PHE 381, TYR 385, TRP 387, MET 522, VAL 523, ALA 527, LEU 531 H-Bond-TYR 385, SER 530 Pi-Pi Stacking- TYR 385, TRP 387
4	Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1	Hydrophobic- TYR 348, VAL 349, LEU 352, TYR 355, LEU 359, PHE 381, LEU 384, TYR 385, TRP 387, PHE 518, MET 522, VAL 523, ALA 527, PHE 529, LEU 531, LEU 534
5	Eicosanoic Acid	Hydrophobic- MET 113, VAL 116, PHE 205, PHE 209, VAL 228, TYR 348, VAL 349, LEU 352, TYR 355, LEU 359, ILE 377, PHE 381, TYR 385, TRP 387, PHE 518, MET 522, VAL 523, ALA 527, PHE 529, LEU 531, LEU 534

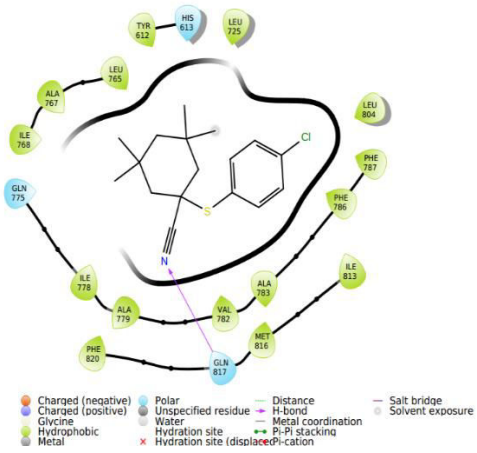
Table 3 is the results of glide docking analysis of receptor Phosphodiesterase type 5 (**PDE5**) inhibitor (1XOZ). The result showed that phytochemical 1(1_Phenyl_2_ phenylsulfonylaminoethyl) aziridine had the best docking score of -9.433 kcal/mol with glide energy, glide Emodel and MMGBSA of -39.276 kcal/mol, -56.35 and -38.37 kcal/mol respectively. However, the receptor's co-crystallized ligand (CIA- Tadalafil) had the best MMGBSA of -52.82 kcal/mol, although with a glide energy (-39.071 kcal/mol) and docking score (-9.027) similar to that of 1(1_Phenyl_2_ phenylsulfonylaminoethyl) aziridine.

TABLE 3: RECEPTOR- Phosphodiesterase type 5 inhibitor (PDE5 inhibitor)

S.No	Compound ID	PUBCHEM ID	Glide Docking Score (kcal/mol)	Glide Energy (kcal/mol)	Glide Emodel	MMGBSA dG Bind (kcal/mol)
1	1_(1_Phenyl_2_ phenylsulfonylaminoethyl)aziridine	583292	-9.433	-39.276	-56.35	-38.37
2	Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1	572764	-9.318	-27.018	10000	-29.76
3	Yohimbine	8969	-9.314	34.031	-44.401	-40.52
4	Tadalafil (Standard)	110635	-9.027	-39.071	10000	-50.62
5	9_12_15_octadecatrienoic acid.1	5280934	-8.962	-48.592	-56.968	-40.17
6	Ajmalicine.1	441975	-8.955	-34.974	-42.514	-41.65
Other PDE5 INHIBITOR STANDARDS						
7	Sildenafil.1	135398744	-8.983	-55.805	-84.621	-63.01
8	Avanafil.1	9869929	-10.455	-59.756	-97.125	-45.04

The 2D protein- ligand interaction of receptor 1XOZ with hit phytochemicals of Rauwolfia vomitoria was as shown in Figure 2. From the figure, amino acids of the receptor had hydrophobic, hydrogen- bond and Pi-Pi stacking interactions with the phytochemicals as detailed in Table 4. Tadalafil had 2 Pi-Pi stacking interactions with amino acids PHE 786 and PHE 820 in addition to the hydrophobic interactions.

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ILE 813
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Glycine
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Metal
Polar
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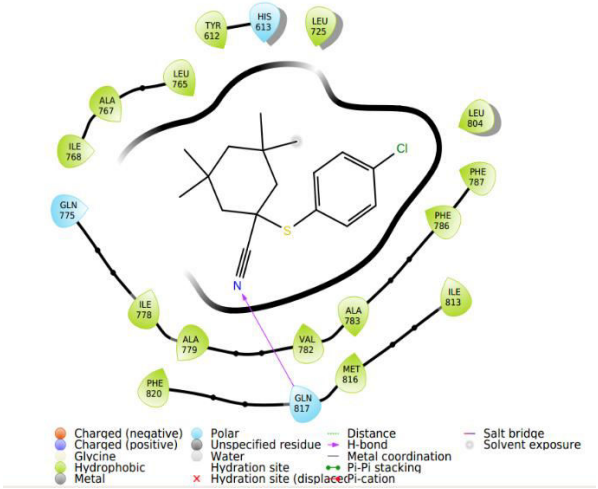
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

C **D**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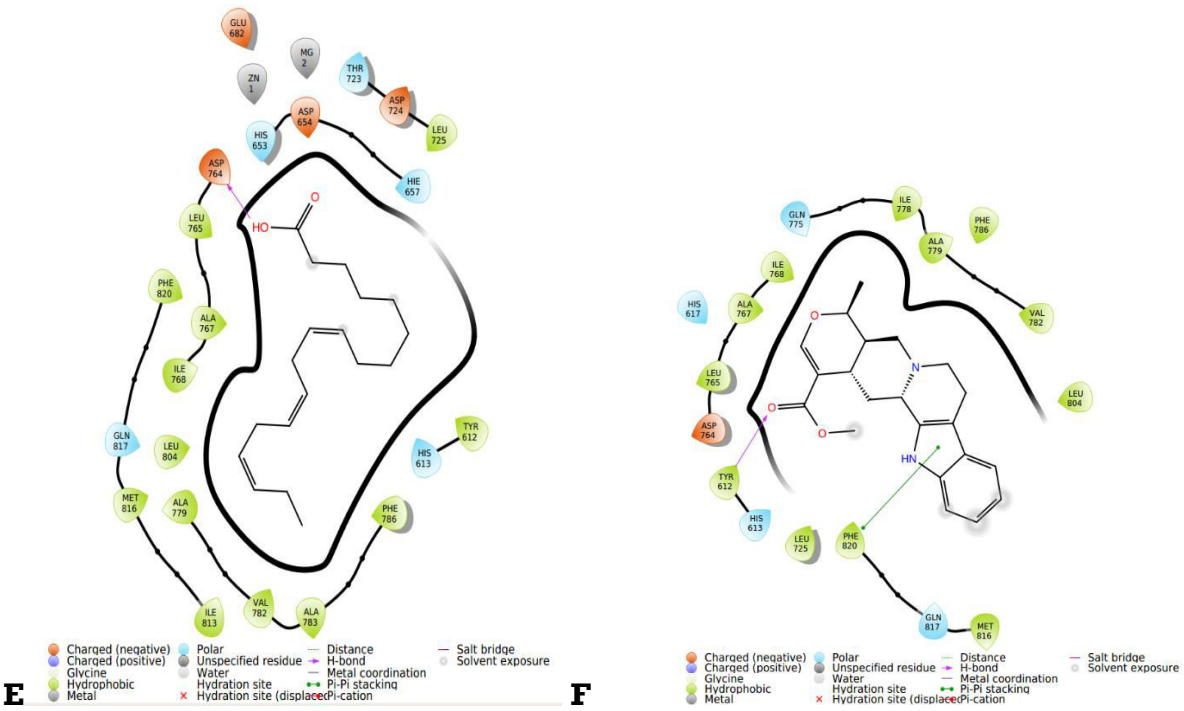

Figure 2: Two-dimensional (2D) molecular interactions of amino acid residues of Phosphodiesterase type 5 (PDE5) inhibitor (1XOZ) with hit compounds of Rauwolfia vomitoria (A) Tadalafil (B) 1(1_Phenyl_2_phenylsulfonylaminoethyl) aziridine (C) Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1 (D) Yohimbine (E) 9_12_15_octadecatrienoic acid.1 (F) Ajmalicine.1

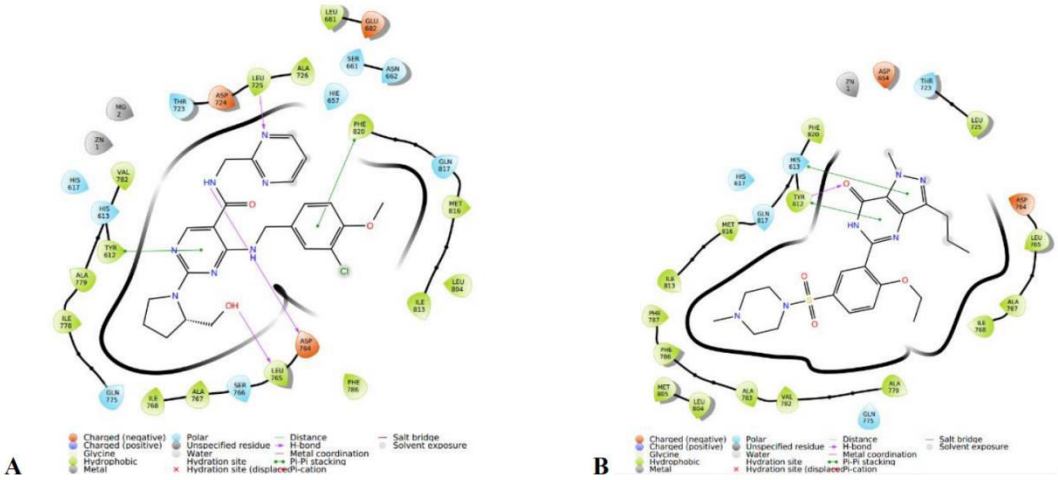

Figure 3: Two-dimensional (2D) molecular interactions of amino acid residues of Phosphodiesterase type 5 (PDE5) inhibitor (1XOZ) with other PDE5 inhibitor(A) Avanafil (B) Sildenafil

Table 4: Phosphodiesterase type 5 inhibitor (PDE5 inhibitor) ligand interaction with hit compounds

S.No	Compound ID	Interacting amino acids
1	1_(1_Phenyl_2_ phenylsulfonylaminoethyl)aziridine	Hydrophobic-LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, ALA 783, PHE 786, ILE 813, MET 816, PHE 820
2	Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1	Hydrophobic-TYR 612, LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, ALA 783, PHE 786, PHE 787, ILE 813, MET 816, PHE 820 H-Bond- GLN 817
3	Yohimbine	Hydrophobic-TYR 612, LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, ALA 783, PHE 786, PHE 787, ILE 813, MET 816, PHE 820 H-Bond- GLN 817
4	CIA- Tadalafil (Standard)	Hydrophobic-TYR 612, LEU 725, LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, PHE 786, MET 816, PHE 820 H-Bond- TYR 612 Pi-Pi stacking- PHE 786, PHE 820
5	9_12_15_octadecatrienoic acid.1	Hydrophobic-TYR 612, LEU 725, LEU 765, ALA 767, ILE 768, ALA 779, VAL 782, ALA 783, PHE 786, ILE 813, MET 816, PHE 820 H-Bond- ASP 764
6	Ajmalicine.1	Hydrophobic-TYR 612, LEU 725, LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, PHE 786, MET 816, PHE 820 H-Bond- TYR 612 Pi-Pi stacking-PHE 820

Table 5 profile the physicochemical and ADMET property of the hit ligands of both receptors used in this study. Properties evaluated includes number of hydrogen bond acceptors, number of hydrogen bond donors, Blood-Brain Barrier Penetration, hERG (human ether-a-go-go related gene), Clearnce,human hepatotoxicity, Drug-induced liver injury, Human Intestinal Absorption, logP and logS.

Table 5: Physicochemical and ADMET Property

Parameters	M1	M2	M3	M4	M5	M6	M7	M8	M9
Pubchem ID	151166	85014	10467	583292	572764	8969	110635	5280934	441975
M.F	$C_{15}H_{13}ClFNO_2$	$C_{21}H_{44}O$	$C_{20}H_{40}O_2$	$C_{16}H_{18}N_2O_2S$	$C_{17}H_{22}ClNS$	$C_{21}H_{26}N_2O_3$	$C_{22}H_{19}N_3O_4$	$C_{18}H_{30}O_2$	$C_{21}H_{24}N_2O_3$
M.W	293.06	312.34	312.3	302.11	307.12	354.19	389.14	278.22	352.18
nHA	3	1	2	4	1	5	7	2	5
nHD	2	1	1	1	0	2	1	1	1
BBB	Negative	Negative	Negative	Positive	Positive	Positive	Negative	Negative	Positive
hERG	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active
CL	Low	Moderate	Low	Low	Low	Moderate	Moderate	Low	Moderate
H-HT	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Negative	Positive
DILI	Toxic	Non-Toxic	Non-Toxic	Toxic	Toxic	Non-Toxic	Toxic	Non-Toxic	Toxic
AMES	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
Carcinogenicity	Non-carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens	Carcinogens	Non-carcinogens	Carcinogens	Carcinogens	Carcinogens
Ro5	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted
SAscore	2.041	1.484	1.665	2.159	2.955	3.875	3.278	2.589	3.936
HIA	0.004	0.004	0.005	0.009	0.024	0.009	0.005	0.026	0.005
logP	4.49	9.045	8.389	2.409	5.584	2.71	3.44	3.147	3.958
logS	-5.24	-6.976	-6.259	-1.698	-6.3	-2.88	-6.003	-3.142	-3.11

M.F-Molecular Formular, M.W- Molecular Weight, nHA- Number of hydrogen bond acceptors, nHD- Number of hydrogen bond donors, BBB-Blood-Brain Barrier Penetration, **hERG** -human ether-a-go-go related gene, CL-Clearance, **H-HT-** human hepatotoxicity, **DILI-** Drug-induced liver injury, **AMES-** test for mutagenicity, Ro5- Lipinski Rule, **SAscore** - Synthetic accessibility score, **HIA-** Human Intestinal Absorption, **logP** - Log of the octanol/water partition coefficient, **logS-** Log of the aqueous solubility. LUR_Lumiracoxib-**M1**, Heneicosanol-**M2**, Eicosanoicacid-**M3**, 1_(1_Phenyl_2_phenylsulfonylaminoethyl)aziridine-**M4**, Cyclohexanecarbonitrile,1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1 -**M5**, Yohimbine- **M6**, CIA-Tadalafil- **M7,**9_12_15_octadecatrienoic acid.1-**M8**, Ajmalicine.1- **M9**

Discussion

The phytochemical compounds of Rauwolfia vomitoriainteracted with COX-2 inhibitor and PDE5 inhibitor with various levels of binding affinity. For each of the receptors (PDB ID 4RRW, 1XOZ), after the virtual screening of the 106 phytochemicals the docking scores were used to rank the compounds and ligands that have glide docking scores close to that of the native ligand were selected for further analysis.

Table 1 shows the binding affinities of the top four scoring compounds and the standard ligand (LUR) with COX-2 Inhibitor (PDB ID: 4RRW). Lumiracoxib (LUR) scored the least with a binding energy of -10.981Kcal/mol followed by Heneicosanol with the binding energy -9.759Kcal/mol, 1(1_Phenyl_2_ phenylsulfonylaminoethyl) aziridine with -9.445 Kcal/mol, Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-1 with -9.157 Kcal/molwhile the scored of Eicosanoic acid was -8.955Kcal/mol. However, for the docking score of PDE5 inhibitor target (PDB ID: 1XOZ) on Table 3, 1(1_Phenyl_2_ phenylsulfonylaminoethyl)-aziridine had the best binding affinity of -9.433 Kcal/mol, followed by Cyclohexanecarbonitrile,-1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-1 with -9.318 Kcal/mol, Yohimbine with -9.314 Kcal/mol, the native ligand CIA (Tadalafil) with -9.027 Kcal/mol, 9_12_15_octadecatrienoic acid.1 with -8.962 Kcal/mol and Ajmalicine.1 with -8.955 Kcal/mol. While the docking score shows the drug molecules potential and their hydrogen bond interaction at the binding site of target, the MM/GBSA method is a more reliable approach frequently used to estimate the binding free energy (BFE) of a small molecule to a protein and this has become a very useful in silico methods for assessing binding affinity (Oluyemi et al., 2022). The BFE provides distinct energy contributions within the binding pockets and the binding conformations that present the best intermolecular interactions at the active sites of the protein (Ryckaert et al., 1977). Evaluating the BFE as reported in Table 1 and Table 3, for the COX-2 inhibitor the BFE of the native ligand was the least with -52.82 Kcal/mol which can suggest the reason for the least docking score too. This was followed by Heneicosanol with MMGBSA of -51.01kcal/mol indicating the potential ability of this compound to compete with LUR as COX-2 inhibitor. However, to emphasis the use MMGBSA for a better decision on drug likeness, Cyclohexanecarbonitrile,-1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-1 with had a docking score of -9.157 Kcal/mol which was ranked higher than Eicosanoic acid with -8.955Kcal/mol but the MMGBSA result indicated that Eicosanoic acid has better BFE of -20.84kcal/mol than 3.12kcal/mol observed for Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-1 suggesting that ligand Eicosanoic acid has more potential to inhibit COX-2 and consequently act effectively as an anti-inflammatory in inflammation related male infertility . This same trend was observed for the phytochemicals docked on PDE5 inhibitor target, with the native ligand (Tadalafil), Ajamalicine, Yohimbine, and 9_12_15_octadecatrienoic acid.1 having a lower BFE of -50.62, -41.65, -40.52, -40.17 kcal/mol than 1(1_Phenyl_2_ phenylsulfonylaminoethyl)-aziridine (-38.37 kcal/mol) with the least docking score. This was also observed when other standard PDE5 inhibitors; Sildenafil and Avanafil was docked in the same pocket. Avanafil has the docking score of -10.455 kcal/mol while Sildanafil has -8.983 kcal/mol. However, the BFE of Sildenafil was -63.01kcal/mol compared to -45.04 kcal/mol of Avanafil. This is indicative of the potential of these selected compounds from R. vomitoria as potential

PDE5 inhibitor. The MM/GBSA method has been applicable in reproducing and rationalising experimental findings and expanding on results from virtual screening and docking calculations. The four major PDE5 inhibitors are sildenafil (Viagra), tadalafil (Cialis), vardenafil (Levitra), and avanafil (Stendra). PDE5 inhibitors are selective, competitive, and reversible, generally working to decrease cGMP metabolism and ultimately leading to successful attainment and maintenance of an erection (Nguyen et al., 2017).

Figures 1 shows the two-dimensional (2D) representations of the molecular interactions of amino-acid residues of Cyclooxygenase-2 (COX-2) Inhibitor (4RRW) with hit compounds of Rauwolfia vomitoria. There were four compounds (Heneicosanol, 1(1_Phenyl_2_ phenylsulfonylaminoethyl)-aziridine, Cyclohexanecarbonitrile,-1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-1, Eicosanoic acid) and the native ligand (LUR). They all interacted with TYR 348, VAL 349, LEU 352, TYR 355, TYR 385, TRP 387, MET 522, VAL 523, ALA 527, LEU 531in addition to other amino acid residues as also recorded on Table 2. LUR formed hydrogen bond with TYR385 and SER530 (Figure 1(A)) as also seen with 1_Phenyl_2_ phenylsulfonylaminoethyl)-aziridine (Figure 1(B)) and Heneicosanol with ARG120 and TYR355(Figure 1(C)). Pi-Pi stacking with TYR385 and TRP 387 was also observed in 1_Phenyl_2_ phenylsulfonylaminoethyl)-aziridine (Table 2). The binding interaction of these Rauwolfia vomitoria compounds with the amino acid residues of the native ligand therefore makes them possible inhibitors of COX-2 and potential anti-inflammatory agents. There have been claims of phytochemicals from Rauwolfia vomitoria having anti-inflammatory potentials (Al, 2015), (Ajayi, 2021).

The two-dimensional (2D) molecular interactions of amino acid residues of Phosphodiesterase type 5 (PDE5) inhibitor (1XOZ) with five selected compounds (1(1_Phenyl_2_phenylsulfonylaminoethyl)-aziridine, Cyclohexanecarbonitrile,1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1, Yohimbine, 9 _12_15_octadecatrienoic acid.1 and Ajmalicine.1) of Rauwolfia vomitoria and Tadalafilwas as shown in Figure 2. There were common interaction with some of the amino acids across the compounds. LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, MET 816 and PHE 820 interacted with all the selected phytocompounds. Tadalafil has hydrogen bond with TYR612 and Pi-Pi stacking with PHE820 (Figure 2(A)) which Ajmalicine also have the same interaction (Figure 2(E)). Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1, and Yohimbine also had hydrogen bond interaction with GLN817 (Figure 2(C,D)) but 9_12_15_octadecatrienoic acid.1 with ASP764 (Figure 2(E)) These were also presented on Table 4. When these were compared with standard PDE5 Avanafil and Sidenafil, they interacted also the same amino acid as shown in Figure 3 indicating that the amino acids reported here are responsible for the inhibitory action of the PDE5 inhibitor target. Avanafil also had Pi-

Pi stacking interaction with TYR612, PHE 820 and hydrogen bond with LEU725, ASP764, LEU765 (Figure 3(A)) and Sidenafil having Pi-Pi stacking interaction with TYR612, HIS613 and hydrogen bond with TYR612. With the selected phytochemicals from Rauwolfia vomitoria having similar amino acids interactions with the native ligand Tadalafil and with Avanafil and Sildenafil, this suggested that these compounds have the potentials of improving erectile function through the mechanism of actions of PDE5, Yohimbine and Ajmalicine obtained from other natural sources have previously been linked to have PDE5 inhibitory property (Singh et al., 2020), (Tettevi et al., 2024).

ADMET analysis is an important step in drug design that is required for evaluating the pharmacokinetics, druglike properties and toxicity behavior of test compounds. The prediction of ADMET properties through in silico determination is a fast and cost-effective alternative to standard experimental methods (Esther et al., 2017) and its early introduction in the drug development process is necessary for minimising the rate of drug failure during pharmacokinetics studies in the clinical phases. Among the selected Rauwolfia vomitoria compounds that water solubility (logS) was predicted for only 1_(1_Phenyl_2_phenylsulfonylaminoethyl)aziridine (-1.698), Yohimbine (-2.88), Ajmalicine.1(-3.11) and 9_12_15_octadecatrienoic acid.1(-3.142) were within the range of -4 to 0.5 log mol/L considered as proper on the Admetlab 2.0. Also for logP only 1_(1_Phenyl_2_phenylsulfonylaminoethyl)aziridine(2.409) andYohimbine (-2.71) are within the range of 0 to 3 log mol/L considered proper (Xiong et al., 2021). However, the native ligands LUR (COX-2 inhibitor) has -5.24 and 4.49 for logS and logP respectively, while for Tadalafil (PDE5 inhibitor) the values are logS(-6.003) and logP (3.44). These indicated that the native ligands likewise were not really water soluble and lipophilic. Water solubility enhances the movement of drug molecules through the aqueous blood when ingested. Although high logP was implicated in reducing hydrophobic binding to macromolecules, including the target receptor(Xiong et al., 2021) the native compounds and the selected phytochemicals were able to have this interaction therefore able to elicit appropriate mechanism of action. The rule of thumb of Lipinski states that orally administered drugs should possess a molecular weight under 500g/mol, 10 or fewer hydrogen bond acceptors, 5 or fewer hydrogen bond donors and a log P less than 5. Any drug molecule that violates two or more of the rules would not be orally active (Lipinski,et al, 1997).

The evaluation of the rule of 5 (Ro5) in this study showed that all selected phytochemicals passed the Ro5.

The human intestinal absorption of an oral drug is the essential prerequisite for its apparent efficacy and the close relationship between oral bioavailability and intestinal absorption has also been proven and HIA can be seen an alternative indicator for oral bioavailability to some extent. It is therefore of interest that all the

phytochemicals have excellent intestinal absorption (Esther et al., 2017). While some of the hit phytochemicals were positive to blood brain barrier penetration, liable to cause drug-induced liver injury and drug induced hepatotoxicity, most are negative to Ames test for mutagenicity. Many of the hit compounds on the PDE5 inhibitor also shows tendency of carcinogenicity. Clearance is also an important pharmacokinetic parameter that defines, together with the volume of distribution, the half-life, and thus the frequency of dosing of a drug (Tettevi et al., 2024), when measured in this study the clearance of the hit compounds was between low to moderate indicating the tendency to stay longer in the body. Therefore, structural modification or optimisation of the lead molecules may be necessary to remove the toxic tendencies shown by some of the compounds while maintaining the COX-2 and PDE5 inhibitory activity.

Conclusion

One hundred and six bioactive compounds of Rauwolfia vomitoria were subjected to virtual screening using molecular docking and ADME studies for the discovery of potential inhibitors of Cyclooxygenase-2 (COX-2) Inhibitor and Phosphodiesterase type 5 inhibitor (PDE5)target. The investigated compounds demonstrated varying levels of binding affinity towards the targets. After the preliminary screening, four compounds were selected for the COX-2 inhibitor target (Heneicosanol, 1(1_Phenyl_2_phenylsulfonylaminoethyl)-aziridine, Cyclohexanecarbonitrile,-1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-1, Eicosanoic acid) and further analysis were performed alongside the native ligand (LUR). Although LUR had the least docking score (-10.981), Heneicosanol followed it with -9.759 and compete actively with a close binding free energy and similar amino acid interaction at the pocket site. However, Heneicosanol had better physicochemical and ADMET property than LUR making it a potential anti-inflammatory compound through the mechanism of Cyclooxygenase-2 (COX-2) Inhibitor. On the other hand, five compounds 1(1_Phenyl_2_phenylsulfonylaminoethyl)-aziridine, Cyclohexanecarbonitrile,1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1, Yohimbine, 9 _12_15_octadecatrienoic acid.1 and Ajmalicine.1 were selected on the Phosphodiesterase type 5 (PDE5) inhibitor and they compete relatively with the co-crystallized ligand (Tadalafil) as some have docking score lower than that of Tadalafil, have comparative MMGBSA and relative ADMET analysis.

However, based on the predicted ADMET outputs of the compounds, optimization of the lead may be needed to improve the drug properties for optimal results. This could be followed wet laboratory experiments and subsequently the development of new cyclooxygenase-2 (COX-2) Inhibitor and Phosphodiesterase type 5 (PDE5) inhibitor candidates which could help in reducing inflammation leading to infertility in male and improve erectile function.

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Table 1: Extra Precision Glide Docking of Cyclooxygenase-2 (COX-2) Inhibitor

S.No	**Compound ID**	**PUBCHEM ID**	**Glide Docking Score** (kcal/mol)	**Glide Energy (kcal/mol)**	**Glide Emodel**	**MMGBSAdG Bind (kcal/mol)**
1	LUR_Lumiracoxib	151166	-10.981	-44.409	-67.667	-52.82
2	Heneicosanol	85014	-9.759	-37.619	-51.228	-51.01
3	1_(1_Phenyl_2_ phenylsulfonylaminoethyl) aziridine	583292	-9.445	-39.272	-55.869	-28.23
4	Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1	572764	-9.157	-14.754	10000	3.12
5	Eicosanoic Acid	10467	-8.955	-33.722	-17.042	-20.84

TABLE 2: Ligand interaction of Cyclooxygenase-2 (COX-2) Inhibitor with selected Phytochemicals from Rauwolfia vomitoria		
S.No	**Compound ID**	**Interacting amino acids**
1	LUR_Lumiracoxib	Hydrophobic-VAL 344, TYR 348, VAL 349, LEU 352, TYR 355, PHE 381, LEU 384, TYR 385, TRP 387, MET 522, VAL 523, GLY 526, ALA 527, LEU 531 H-Bond- TYR 385, SER 530
2	Heneicosanol	Hydrophobic- PHE 205, PHE 209, VAL 228, VAL 344, TYR 348, VAL 349, LEU 352, TYR 355,ILE 377, LEU 384, TYR 385, TRP 387, PHE 518,MET 522, VAL 523, ALA 527, LEU 531, LEU 534 H-Bond- ARG 120, TYR355
3	1(1_Phenyl_2_ phenylsulfonylaminoethyl) aziridine	Hydrophobic- TYR 348, VAL 349, LEU 352, TYR 355, PHE 381, LEU 384, PHE 381, TYR 385, TRP 387, MET 522, VAL 523, ALA 527, LEU 531 H-Bond-TYR 385, SER 530 Pi-Pi Stacking- TYR 385, TRP 387
4	Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1	Hydrophobic- TYR 348, VAL 349, LEU 352, TYR 355, LEU 359, PHE 381, LEU 384, TYR 385, TRP 387, PHE 518, MET 522, VAL 523, ALA 527, PHE 529, LEU 531, LEU 534
5	Eicosanoic Acid	Hydrophobic- MET 113, VAL 116, PHE 205, PHE 209, VAL 228, TYR 348, VAL 349, LEU 352, TYR 355, LEU 359, ILE 377, PHE 381, TYR 385, TRP 387, PHE 518, MET 522, VAL 523,

		ALA 527, PHE 529, LEU 531, LEU 534

TABLE 3: RECEPTOR- Phosphodiesterase type 5 inhibitor (PDE5 inhibitor)

S.No	Compound ID	PUBCHEM ID	Glide Docking Score (kcal/mol)	Glide Energy (kcal/mol)	Glide Emodel	MMGBSA dG Bind (kcal/mol)
1	1_(1_Phenyl_2_ phenylsulfonylaminoethyl)aziridine	583292	-9.433	-39.276	-56.35	-38.37
2	Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1	572764	-9.318	-27.018	10000	-29.76
3	Yohimbine	8969	-9.314	34.031	-44.401	-40.52
4	Tadalafil (Standard)	110635	-9.027	-39.071	10000	-50.62
5	9_12_15_octadecatrienoic acid.1	5280934	-8.962	-48.592	-56.968	-40.17
6	Ajmalicine.1	441975	-8.955	-34.974	-42.514	-41.65
Other PDE5 INHIBITOR STANDARDS						
7	Sildenafil.1	135398744	-8.983	-55.805	-84.621	-63.01
8	Avanafil.1	9869929	-10.455	-59.756	-97.125	-45.04

Table 4: Phosphodiesterase type 5 inhibitor (PDE5 inhibitor) ligand interaction with hit compounds		
S.No	Compound ID	Interacting amino acids
1	1_(1_Phenyl_2_ phenylsulfonylaminoethyl)aziridine	Hydrophobic-LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, ALA 783, PHE 786, ILE 813, MET 816, PHE 820
2	Cyclohexanecarbonitrile, 1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1	Hydrophobic-TYR 612, LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, ALA 783, PHE 786, PHE 787, ILE 813, MET 816, PHE 820 H-Bond- GLN 817
3	Yohimbine	Hydrophobic-TYR 612, LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, ALA 783, PHE 786, PHE 787, ILE 813, MET 816, PHE 820 H-Bond- GLN 817
4	CIA- Tadalafil (Standard)	Hydrophobic-TYR 612, LEU 725, LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, PHE 786, MET 816, PHE 820 H-Bond- TYR 612 Pi-Pi stacking- PHE 786, PHE 820
5	9_12_15_octadecatrienoic acid.1	Hydrophobic-TYR 612, LEU 725, LEU 765, ALA 767, ILE 768, ALA 779, VAL 782, ALA 783, PHE 786, ILE 813, MET 816, PHE 820 H-Bond- ASP 764

Table 5: Physicochemical and ADMET Property

6	Ajmalicine.1	Hydrophobic-TYR 612, LEU 725, LEU 765, ALA 767, ILE 768, ILE 778, ALA 779, VAL 782, PHE 786, MET 816, PHE 820 H-Bond- TYR 612 Pi-Pi stacking-PHE 820

Parameters	**M1**	**M2**		**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	
Pubchem ID	151166	85014		10467	583292	572764	8969	110635	528034	441975	
M.F	$C_{15}H_{13}ClFNO_2$	$C_{21}H_{44}O$		$C_{20}H_{40}O_2$	$C_{16}H_{18}N_2O_2S$	$C_{17}H_{22}ClNS$	$C_{21}H_{26}N_2O_3$	$C_{22}H_{19}N_3O_4$	$C_{18}H_{30}O_2$	$C_{21}H_{24}N_2O_3$	
M.W	293.06	312.34		312.3	302.11	307.12	354.19	389.14	278.22	352.18	
nHA	3	1		2	4	1	5	7	2	5	
nHD	2	1		1	1	0	2	1	1	1	
BBB	Negative	Negative		Negative	Positive	Positive	Positive	Negative	Negative	Positive	
hERG	Inactive	Inactive		Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	
CL	Low	Moderate		Low	Low	Low	Moderate	Moderate	Low	Moderate	
H-HT	Positive	Negative		Negative	Positive	Positive	Negative	Positive	Negative	Positive	
DILI	Toxic	Non-Toxic		Non-Toxic	Toxic	Toxic	Non-Toxic	Toxic	Non-Toxic	Toxic	
AMES	Negative	Negative		Negative	Negative	Negative	Negative	Negative	Positive	Positive	
Carcinogenicity	Non-carcinogens	Non-carcinogens		Non-carcinogens	Non-carcinogens	Carcinogens	Non-carcinogens	Carcinogens	Carcinogens	Carcinogens	
Ro5	Accepted	Accepted		Accepted	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted	
SAscore	2.041	1.484		1.665	2.159	2.955	3.875	3.278	2.589	3.936	
HIA	0.004	0.004		0.005	0.009	0.024	0.009	0.005	0.026	0.005	
logP	4.49	9.045		8.389	2.409	5.584	2.71	3.44	3.147	3.958	
logS	-5.24	-6.976		-6.259	-1.698	-6.3	-2.88	-6.003	-3.142	-3.11	
M.F-Molecular Formular, M.W- Molecular Weight, nHA- Number of hydrogen bond acceptors, nHD- Number of hydrogen bond donors, BBB- Blood-Brain Barrier											

Penetration, **hERG** -human ether-a-go-go related gene, CL-Clearance, **H-HT-** human hepatotoxicity, **DILI-** Drug-induced liver injury, **AMES-** test for mutagenicity, Ro5-Lipinski Rule, **SAscore** - Synthetic accessibility score, **HIA-** Human Intestinal Absorption, **logP** - Log of the octanol/water partition coefficient, **logS-** Log of the aqueous solubility.

LUR_Lumiracoxib-**M1**, Heneicosanol-**M2**, Eicosanoicacid-**M3**, 1_(1_Phenyl_2_phenylsulfonylaminoethyl)aziridine-**M4**, Cyclohexanecarbonitrile,1-[(4-chlorophenyl)thio]-3,3,5,5-tetramethyl-.1 **-M5**, Yohimbine- **M6**, CIA-Tadalafil-**M7,**9_12_15_octadecatrienoic acid.1-**M8**, Ajmalicine.1- **M9**