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SITE-SPECIFIC DOCKING AGAINST HIV- RT PROTEIN USING AUTODOCK 4.2.6

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ABSTRACT

HIV is one of the most austere and deadly infectious viruses with disastrous concerns. Moreover, it is a significant global public health concern with rising rates of morbidity and mortality despite antiretroviral medication availability. However, the majority of anti-HIV-1 medications specifically target reverse transcriptase (RT) to prevent the synthesis of cDNA and the subsequent chain of events that lead to retroviral replication. In this study, site-specific protein-ligand docking was performed in virtual box 6.1 using AutoDock 4.2.6 to determine phytochemicals that can target the HIV-RT protein (PDB ID:1REV). A clinical trial drug (delavirdine) was provided as the standard, and 25 phytochemicals were chosen. Furthermore, the genetic algorithm runs were set to 150 and when the docking grid box was generated the x, y, and z values were 11.96, -21.505, and 25.36. BIOVIA DS and chimera were used to determine the docking poses and interactions. Effective phytochemicals were analyzed based on binding energy (BE) and inhibition constant (Ki), lupeol (BE: -11.15 kcal/mol, Ki:6.67nM), masllinic acid (BE: -11.15 kcal/mol, Ki:6.71nM), withaferin A (BE: -11.15 kcal/mol. Ki:6.70nM) and lupatic acid (BE: -10.59kcal/mol, Ki:17.20nM) respectively. Redocking (0.000Å) was conducted to validate the docking protocol. The common amino acid interactions were observed in LYS 101, LEU 100 and PRO 95 using BIOVIA. disported the most effective phytochemical for treating HIV-1 during the ADMET lab 2.0. Withaferin A and lupeol are expected to have a good bioavailability because of their high cell permeability, absorption of Caco2 and compliance with the Lipinski rule.

Keywords: HIV-reverse transcriptase, 1REV, phytochemicals

INTRODUCTION

The retrovirus HIV causes AIDS in humans. HIV infects CD4+ T-cells, which are important helper T-cells in the human immune system. HIV is a single-stranded, positive-sense RNA virus that is encapsulated. HIV-1 and HIV-2 are the two forms of HIV that have been identified (Phillips et al., 2018). In 1999, scientists discovered a chimp SIV virus that was identical to HIV. According to researchers, in 1920 SIV was initially transmitted to humans in the Congo. More than 70 million people have been infected with HIV, and about 35 million have died of AIDS by the beginning of 2021. Africa continues to be the most badly affected in every 25 adults living with HIV more than two-thirds of all HIV-positive people worldwide (WHO, 2021). However, HIV still represents a significant global epidemic. The lifespan of HIV-positive individuals has risen due to ART (Fig 2). Therefore, ART is vital for survival to reduce morbidity caused by drug toxicity and developing resistance. Therefore, the development of an efficient vaccination is still a distant goal. Sri Lanka has a low-level HIV epidemic, with estimates of HIV prevalence of less than 0.1% which is

lower than most South Asian countries. Mostly from Colombo (60%) and Kandy and Galle (40%) respectively. Mainly, can be seen among young people because of a lack of sex education (Manathunge et al., 2020).

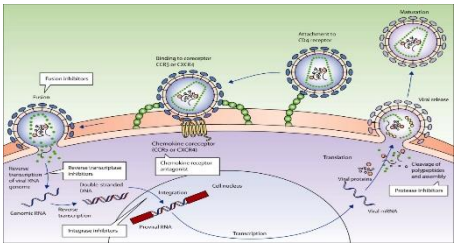


Figure 1: Life cycle of HIV/AIDS (Maartens, Celum and Lewin, 2014)

There are five phases in the HIV cycle. When a CD4 cell is identified, the virus invades it by adhering to receptors on its outer membrane, fusing with the cell, and releasing viral RNA and enzymes. The virus uses an enzyme called reverse transcriptase to reverse-transcribe its single-stranded viral RNA into double-stranded DNA. During integration, the virus interrupts the CD4 cell by integrating its produced viral DNA into the cell's nucleus using the enzyme integrase. Following that replication, the CD4 cell continues the process of producing new virus copies, leading to mutations in the new virions. During budding and maturation, new HIV virions penetrate the CD4 cell's outer membrane. Proportionately, it can devastate the entire immune system (Fig 1) (Maartens, Celum and Lewin, 2014). Furthermore, HIV infection can be transmitted in various ways, although it is most typically done by infected blood or blood components (Laila et al., 2019)

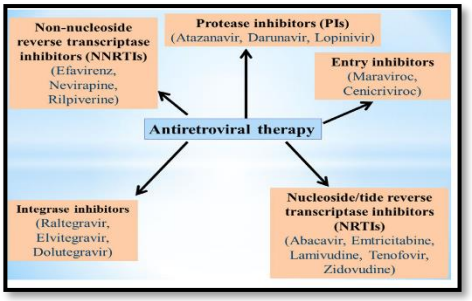


Figure 2: Different antiretroviral regimens and their mode of action (Laila et al., 2019)

Protein-ligand docking is a computational approach that is objective in accurately predicting non-covalent interactions of receptor-ligand binding (Jenwitheesuk and Samudrala, 2003). There are two main techniques: Blind Docking (BD) and Site-Specific Docking (SSD). The technique known as BD involves docking a ligand to the entire protein surface without being aware of the target pocket. Docking a ligand to the active site of a protein knowing the target pocket is known as SSD (Huang and Zou, 2010) (Fig 3). Furthermore, two modes of docking exploration are frequently used: evaluation of docking scores to predict which ligands are likely to bind favourably, and analysis of binding poses to establish which interactions are significant in forming the protein-ligand bond (Lippert and Rarey, 2009).

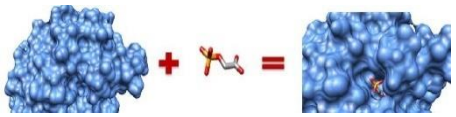


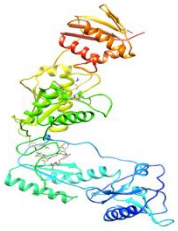
Figure 3: Protein-ligand docking components

In addition to RT, retroviral RNA is annihilated while making cDNA by the larger part (p66) of the HIV-1 RT's ribonuclease hydrogen domain. Most anti-HIV-1 drugs target the RT to prevent

cDNA synthesis and the subsequent sequence of retroviral replication actions (Salehi et al., 2018).

Table 1: Enumeration of receptors

Receptor	Active chain	Experimental data method	Resolution	Natural ligand	Mutations
HIV - 1 RT PDB ID:1REV	A* B	X-ray diffraction	2.60Å	TB 9	No



These FDA-approved drugs are utilized as standard drugs in protein-ligand docking due to their safety and controllability (Thind and Kowey, 2020).

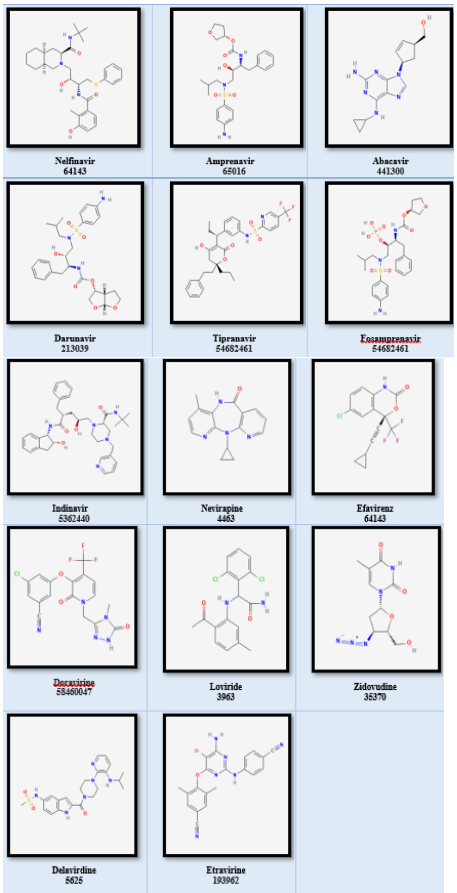


Figure 4: 2D structures and PubChem IDs of FDA-approved drugs (Umaarasu, Padmavathy, Thirunavukkarasu and Gnanendra, 2019) (Ko et al., 2010) (Coman et al., 2007) (Castilho et al., 2018)

Although, natural compounds such as flavonoid, alkaloid, and phenolic compounds have been suggested as potential anti-HIV therapeutics (Salehi et al., 2018) (Fig 5) (Table 2). Therefore, experimental research into innovative HIV therapeutics should be done using ethnomedicines and other natural sources.

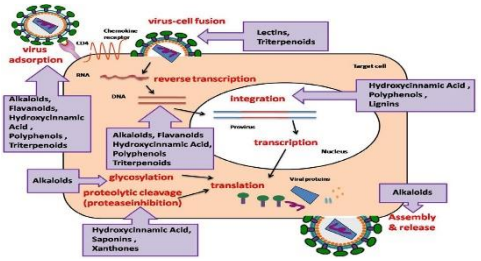
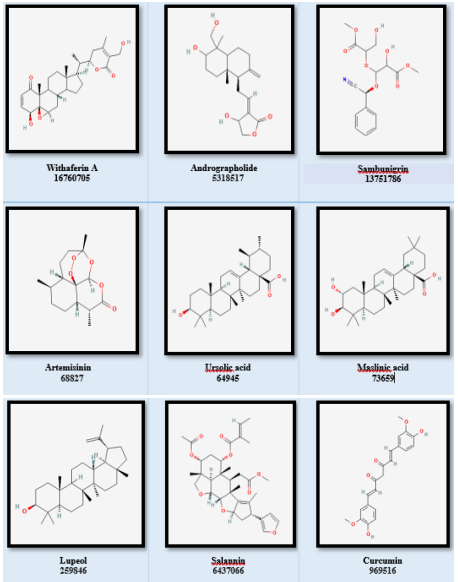


Figure 5: Different phytochemicals and their targets in the HIV replication cycle (Jadaun, Khopkar and Kulkarni, 2016)



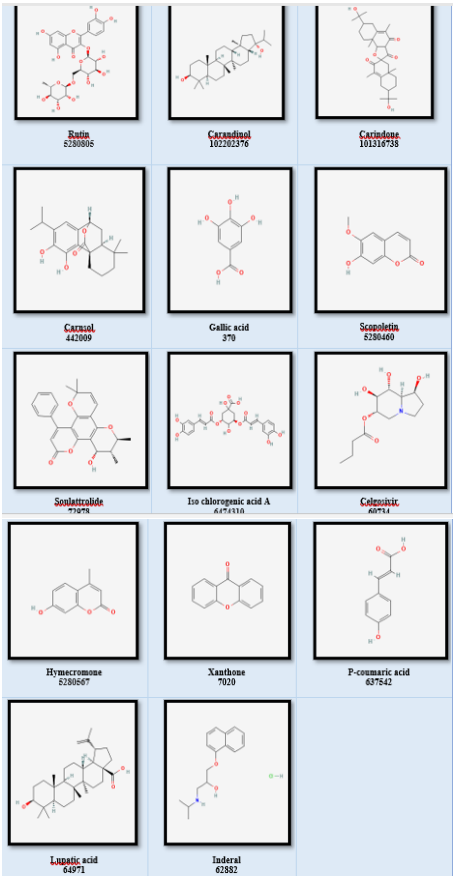


Figure 6: 2D structures and PubChem IDs of phytochemicals (Umaarasu et al., 2019) (Burgos et al., 2020) (Salehi et al., 2018)

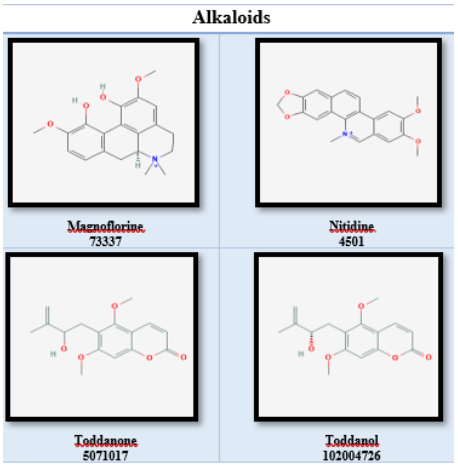


Figure 7: Alkaloids and PubChem IDs

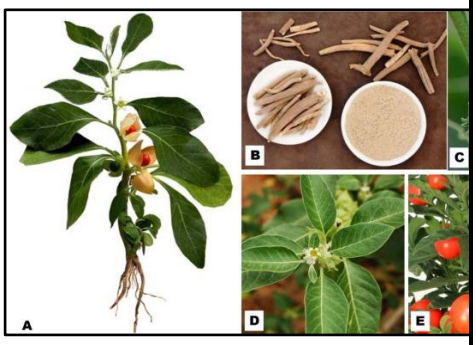
Table 1: List of the phytochemicals (Salehi *et al*., 2018)			
Phytochemical	**Plant name**	**Extracted from**	**Sri Lankan name**
Withaferin A	***Withania somnifera***  **Figure 1:**Withania sominifera plant (A) plant (B) roots and its powder C. Flowers. (D) leaves (E) fruits (Sengupta et al., 2018)	Root	Ashwagandha
Andrographolide	***Andrographis paniculata***  **Figure 2:** Andrographis paniculata plant and leaves	Leaf	Heen Binkohomba
Lupeol **Carindone** **Carnsol** **Gallic acid** **Carindinol** **p- caumaric acid** **Rutin** **Scopoletin** **Ursolic acid**	***Carissa carandas***	Fruit	Maha karamba

	 **Figure 3**:C. carandas plant (A) and its phytochemical products (B – F) (Kumar et al., 2018)		
Artemisinin	***Artemisia annua***  **Figure 4:** Artemisia annua plant and its product (tea)	Leaf	Wal kalandu

AutoDock suite 4.2.6 comprised AD4 and AutoDock vina. In order to reduce the flexibility of the protein, AD4 combines the Lamarckian algorithm and the empirical force field (Forli et al., 2016) and to ease the binding confirmation and free energy associations of the docked complex. In contrast, the ligand data file is converted into the required formats using Open Babel GUI. UCSF Chimera is a high-performance software with a fundamental expansion that facilitates docking visualising, and analysing user data (Butt et al., 2020). BIOVIA DS and LIGPLOT+ are used to visualize the high-quality 2D interactions diagram representing conventional hydrogen and hydrophobic interactions. Also, PyMOL is used for the 3D visualization of docked complex poses. However, the precision of the results is impacted by the speed of the CPU cores (Ritchie and Venkatraman, 2010).

Redocking and Ramachandran plots are used for validation of the docking procedure. In redocking, a cluster tolerance of 2.0 Å in positional RMSD is used to cluster the docking result. The procedure of protein-ligand docking is valid when a natural ligand co-crystal structure is superimposed with a redocked complex whether the RMSD value is less than 2Å (Zubair et al., 2020). In addition, the Ramachandran plot is used to analyze the docking procedure by obtaining percentages of the most favourable region.

Receptor destruction can be estimated by determining the percentages.

The significance of this study is identifying the best ligands and binding sites for HIV-RT receptors to cure HIV/AIDS. Certain HIV drugs have received FDA approval; however, they have low oral bioavailability and a variety of negative effects. But there is still no cure for HIV that is proven to be effective. To overcome these concerns different anti-viral drugs must be developed which should have a high safety profile and pharmacokinetics. As a result, the development of antiretroviral drugs to treat HIV has been extremely beneficial (Simon, Ho and Abdool Karim, 2006). Indicating anti-HIV drugs, natural compounds originating from plants continue to be a rich source of novel treatments. Because bringing a drug to market can take many years and a monumental investment. Hence, researchers had to find new treatments in the most cost-effective possible manner. The discovery of new compounds, binding sites, or conformations, and even the repurposing of a drug to treat HIV. HIV/AIDS is a disease that not only impends physical health but also impacts patients' emotional and social well-being due to negative attitudes and social stigma. In future, researchers able to develop effective phytochemicals or FDA-approved drugs for HIV/AIDS, which will be tremendously beneficial to these patients global (Mohraz et al., 2015).

Objectives

- Protein-ligand docking study for the identification of therapeutic ligands and their binding sites against HIV receptors.
- To familiar with software such as AutoDock/AutoDock Vina, Chimera, PyMOL, LIGPLOT+, Open Babel GUI and BIOVIA DS.
- To identify potent phytochemicals and their binding site against using blind and site-specific Docking.

Materials

The hardware included a Lenovo laptop with Windows 10 and configured with an Intel(R) Core 2, CPU N3060 @ 1.60GHz 64-bit processor, 2GB RAM, and virtual box 6.1.3.4 with a 64-bit processor and 8GB RAM. AutoDock 4.2.6, Open Babel GUI 2.4.1, and BIOVIA Discovery Studio Visualizer 21.1.1.0.0 were the software used. In addition, Python 3.10.0 and MGL Tools 1.5.6 were installed to support the execution of AutoDock 4.2.6. The NCBI PubChem database, the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB), and ADMETlab2.0 were the web tools used. The three-dimensional structure of the HIV-RT protein (PDB ID: 1REV) was received from the PDB while the ligands (Fig 5-8) were acquired from NCBI PubChem.

METHODOLOGY

The pdb file of the receptor was retrieved. The water molecules and chain B were deleted using Autodock 4.2.6. The heteroatom TB9 was eliminated from chain A. Following the addition of the polar hydrogen bonds and the merging of the non-polar and Kollman charges, the missing atoms were identified and repaired. A pdbqt file was used to store this content. The ligands were acquired in. sdf format and Open Babel GUI was used to convert them to a pdbqt file. Gasteiger charges were added and the number of rotatable bonds for each ligand was computed when the file was opened in Autodock 4.2.6. Torsions were set and the ligands were saved in a .pdbqt file. A grid box was created to enclose a specific region of the A chain. The grid dimensions were adjusted as follows; x = 122, y = 122

and z = 112, while the grid center values were x = 11.960, y = -21.505 and z = 25.360and its spacing was 0.375.

The docking search parameter chosen was the Lamarckian genetic algorithm where the genetic algorithm runs were set to 10 with a population size of 150. Subsequently, Autogrid was executed, which produced grid maps for individual atoms of the ligand to be docked. A docking log (DLG) file was produced, containing the binding energies (BE) and inhibitory constant (Ki) values for all the poses generated per ligand. Docking poses were obtained from the BIOVIA discovery 2021 opening docked complex using BIOVIA DS and chimaera 1.16. Selected Options "Publication 1" and "Interactive 1" were used to optimise docking poses. Opening the docked complex allowed LIGPLOT+ to display interactions. It was possible to obtain the 2D interaction plot with HPI and HI. The visualisation of conventional HIs and HPIs (Pi-sigma, Pi-sulfur, alkyl, Pi-alkyl, and Pi-amide) was performed using BIOVIA 2021. The technique was validated by re-docking. The PROCHECK from SAVESv 6.0 was used to validate the re-docked complex by uploading the PDB file. However, the most favourable region was investigated by obtaining percentage differences between dockings before and after. PyMOL 2.5 was utilised to validate the ligand. SSD was applied to the TB9 using the same docking process that was used for the previous ligands. Using PyMOL, the redocked file of the co-crystallized 1REV and TB9 structure was acquired, and the superimposed structure was viewed along with the associated RMSD value. Then, the redocked complex and the receptor's co-crystal structure were examined using LIGPLOT to find the common amino acid interactions. The PubChem database was used to obtain the phytochemicals' canonical SMILEs. The absorption, distribution, metabolism, and excretion (ADMET) parameters were predicted using these in ADMETlab2.0. Their potential drug receptivity was determined using Lipinski's rule of five.

RESULTS

***Table 3:*SSD using AutoDock 4.2.6 results**				
1REV				
FDA – approved drugs				
	Ligand	BFE (kcal/mol)	Ki (nM)	LE
1.	Efavirenz	-10.61	16.57	-0.38
2.	Delavirdine	-9.34	141.97	-0.29
3.	Etravirine	-9.12	207.33	-0.28
4.	Nevirapine	-8.50	592.22	-0.42
5.	Abacavir	-8.08	1190	-0.28
6.	Lovirdine	-7.40	3780	-0.39
7.	Zidovudine	-7.26	4750	-0.38
Phytochemicals				

1.	Lupeol	-11.15	6.67	-0.51
2.	Withaferin A	-11.15	6.7	-0.33
3.	Maslinic acid	-11.15	6.71	-0.36
4.	Lupatic acid	-10.59	17.2	-0.32
5.	Carnsol	-9.74	72.61	-0.3
6.	Carindinol	-9.72	75.62	-0.3
7.	Gallic acid	-9.03	239.98	-0.28
8.	Artemisinin	-8.46	627.66	-0.45
9.	Carindone	-8.39	708.28	-0.23
10.	Sambunigrin	-8.36	750.17	-0.25
11.	Andrographolide	-8.09	1170	-0.32
12.	Xanthone	-7.25	4890	-0.48
13.	Hymecromone	-7.24	5900	-0.48
14.	Toddanone	-7.04	6920	-0.34

Efavirenz was the best anti-HIV-1RT drug among all FDA-approved drugs since it demonstrated the lowest BFE and lowest inhibition constant. Among all the phytochemicals lupeol, maslinic, withaferin A, and lupatic acid were the best potential HIV-1 inhibitors, with BFEs between -11.15 and -10.59 kcal/mol and inhibition constants between 6.67 and 17.2nM respectively.

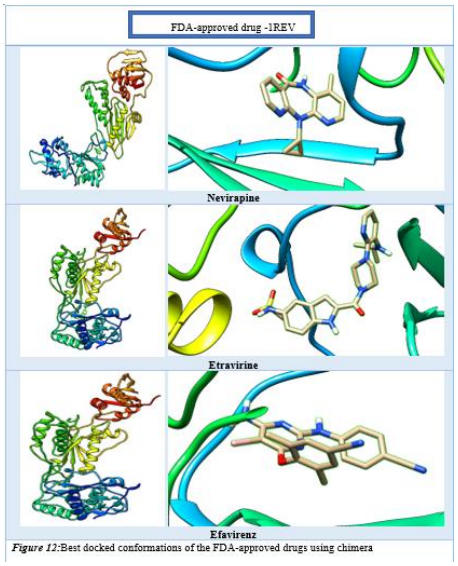


*Figure 12:*Best docked conformations of the FDA-approved drugs using chimera

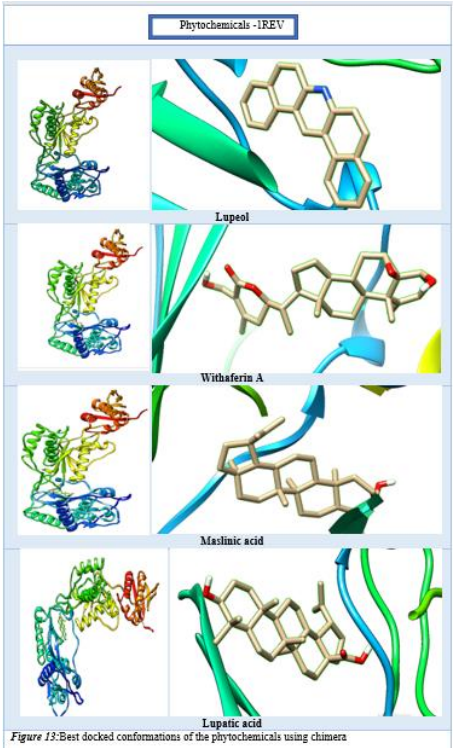


*Figure 13:*Best docked conformations of the phytochemicals using chimera

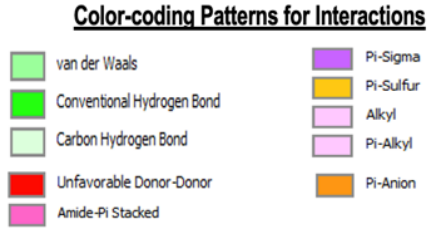


*Table 4:*Interactions of 1REV

	FDA approved drug	Interactions		
		H-bonds	No	Hydrophobic interactions
1.	Efavirenz	PRO236	1	VAL106, TYR188, TYR181, LYS101, LEU100
2.	Delavirdine	HIS96 VAL381 LYS101	3	VAL106, TYR188, LEU100, TYR181, TRP229, GLY99
3.	Etravirine	ILE382 HIS96	2	LEU234, LYS103, VAL106, TYR188, TYR181, VAL179, LYS103, LEU100, LYS101
4.	Nevirapine	-	-	ILE180, LEU234, VAL106, VAL179, TYR181, TYR188, TRP229, PHE227
5.	Abacavir	-	-	VAL106, LYS103, TYR181, LEU100, LYS101, PRO95, TRP229, TYR183
6.	Loviridine	LYS103 LYS101	2	VAL106, TYR318, VAL179, LEU234
7.	Zidovudine	VAL170 GLY190	2	LEU234, VAL106, TYR188, TYR181, TRP229

The common amino acids with respect to HPIs were VAL106 and TYR188. Additionally, the visible amino acid involved in hydrogen bonding was LYS101.

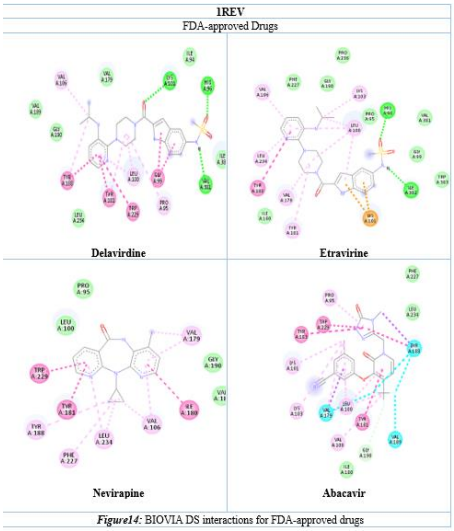


Figure14: BIOVIA DS interactions for FDA-approved drugs

*Table 5:*Interactions of 1REV

	Phytochemical	Interactions		
		HI	No	HPI
1.	Lupeol	-	-	LYS103, LEU100, PRO95, LEU234, VAL106, TYR318
2.	Withaferin A	LYS103 ILE180	2	PHE227, LEU234, TYR188, LEU100, PRO95, TYR181
3.	Maslinic acid	-	-	TRP229, TYR188, PRO95, LEU234, PHE227, VAL106, LEU100, VAL179, TYR181
4.	Lupatic acid	-	-	PHE227, LEU234, VAL106, TYR188, ILE180, VAL179, LEU100, TYR181, TRP229
5.	Carnsol	HIS96	1	LYS103, VAL106, VAL179, LEU100, LEU234
6.	Carindinol	ASN265 SER268	2	LYS353, TRP266, HIS96, TYR232, ILE94
7.	Gallic acid	LYS101 TYR188	2	TRP229, TRY181, VAL179, LEU100, VAL106, PRO95, LEU234
8.	Artemisinin	LYS101 LYS103	3	VAL179, VAL106, LEU100, TYR318
9.	Carindone	PRO243 GLN242	2	LYS263, ILE244, TRP266
10.	Sambunigrin	-	-	LEU234, VAL106, VAL179, TYR181
11.	Andrographolide	-	-	LEU100, VAL106, VAL179
12.	Xanthone	-	-	LEU234, VAL106, TYR188, TRP229
13.	Hymecromone	-	-	LYS103, VAL106, TYR188, LEU234, LEU100, TYR318
14.	Toddanone	-	-	VAL106, LEU234, PHE227, TYR183, TYR181, TYR188, TRP229, LEU100
15.	Toddanol	-	-	VAL106, LYS103, TYR188, LEU234, PHE227, TRP229, PHE227
16.	Isochlorogenic acid A	ILE180 GLN161 TYR188	3	LEU234, TRP229, LEU234
17.	Magnoflorine	LYS103	1	TYR181, LEU100, VAL179, TRP229, VAL106, PRO95, TYR188, LEU234, PHE227
18.	Nitidine	LYS103	1	LEU100, VAL179, LYS101, VAL106, LEU234, PHE227, TYR188, TRP229, TYR181
19.	Rutin	GLN91	1	VAL90, TYR181, TYR183

The common amino acids concerning HPIs were VAL106, LEU100, and LEU234. Additionally, LYS103 was the probable amino acid implicated in hydrogen bonding.

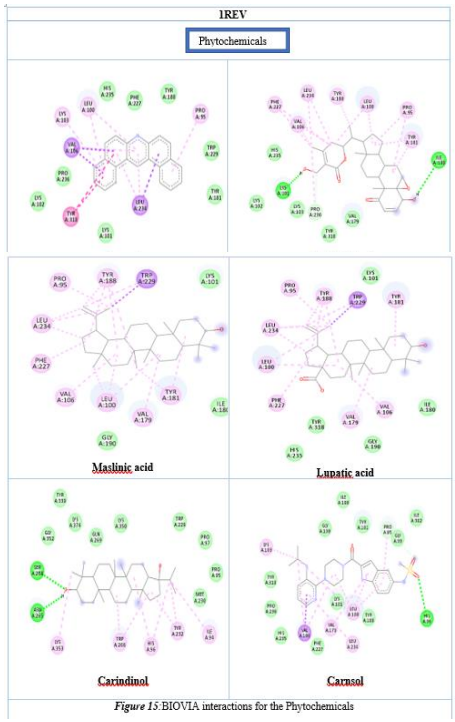


*Figure 15:*BIOVIA interactions for the Phytochemicals

*Table 6:*Comparison of the ADMET properties of the best phytochemicals (Sepehri, Saghaie and Fassihi, 2016)

Phytochemical	MW	αHA	logP	LR	Caco2 permeability	BBB penetration	AM ES	CYP 142	hERG Blockers
Carandinol	444.40	2	6.341	√	- 4.987	√	X	0.1	X
Withaferin A	470.27	6	3.398	√	- 4.981	√	X	0.1	X
Maslinic acid	472.36	4	5.667	√	-5.331	√	X	0.1	X
Lupatic acid	456.36	3	6.005	√	-5.248	√	X	0.1	X
Lupeol	426.39	1	7.291	√	-5.020	√	X	0.1	X
Gallic acid	170.02	5	0.645	√	-5.728	X	X	0.1	X
Carindone	512.310	6	4.080	√	-5.095	√	X	0.1	X
Sambunigrin	295.110	7	0.528	√	-5.306	√	√	0.1	X
Andrographolide	350.210	5	1.580	√	-4.793	√	√	0.1	X
Iso chlorogenic acid A	516.130	12	1.599	√	-6.177	X	X	0.1 – 0.3	X
Artemisinin	262.120	4	1.318	√	-4.695	√	X	0.1	X

*Physicochemical property - MW- Molecular Weight contains hydrogen atoms. Optimal Range (OR): 100-600; αHA- number of hydrogen bond acceptors (OR: 0 -12); logS -Log of the aqueous solubility (OR: -4 - 0.5 logmol/L); logP – lipophilicity (OR: 0 - 5)
Medicinal chemistry - Lipinski rule (LR) is acceptable (OR: MW ≤ 500; logP ≤ 5). Absorption- Caco2 (colon adenocarcinoma cell line) permeability – higher than -5.15log unit; Distribution - BBB – blood brain barrier penetration (√); Toxicity- AMES – probability of being toxic (√), hERG Blockers – the probability of being active (√); Metabolism - CYP 142 – probability of being inhibitor (OR: 0.5 -0.1)

All the ligands acknowledged the five Lipinski rules. Typically, withaferin A had an optimum logP (larger than 1 or lower than 4) while it had greater penetration through biological membranes and greater absorption of gastrointestinal (Namthabad and Mamidala, 2014) (Gao, Gesenberg and Zheng, 2017) that are thought to be more likely to have the best physicochemical and ADME properties for oral therapeutics. Additionally, lupeol, carandinol, and lupatic acid had the highest LogP values of the ligands, indicating a decrease in hydrophobic activity. Additionally, withaferin A had excellent oral bioavailability due to their Furthermore, gallic acid and iso chlorogenic acid A potentially prevent CNS negative effects because they failed to penetrate the BBB. Furthermore, the absence of hERG blockage enables all the phytochemicals to prevent various long QT syndromes and even abrupt death (Wang et al., 2016).

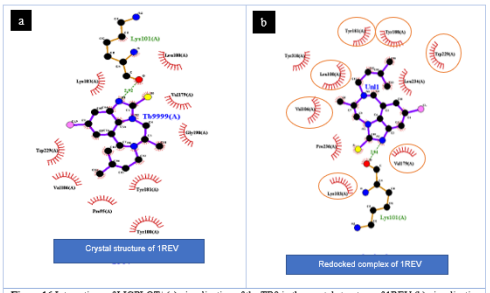


Figure 16: Interactions of LIGPLOT+ (a) visualization of the TB9 in the crystal structure of 1REV (b) visualization of the TB9 in the SSD redocked complex and common amino acids obtained from the crystal structure (*In the red circles)

Furthermore, common HPIs (VAL179, VAL106, TYR181, LEU100, TYR188, and LYS103) were identified in the docked and native 1REV during the AD4 redocking of TB9 (BA: -9.07kcal/mol).

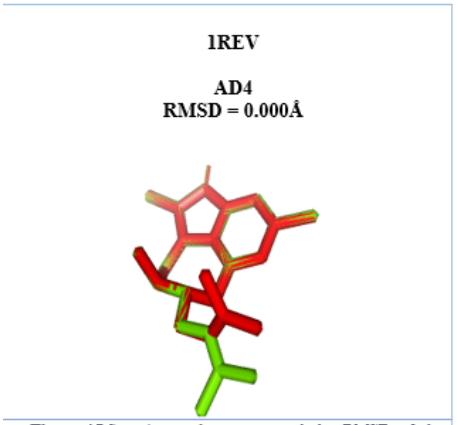


Figure 17: Superimposed structure and the RMSD of the redocked TB9(red) and its native (green) obtained from PyMOL

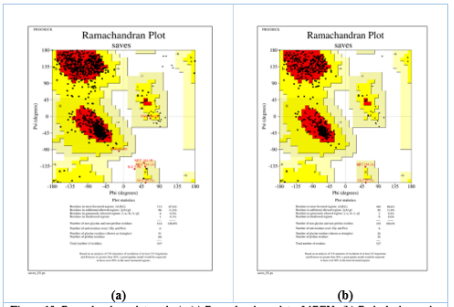


Figure 18: Ramachandran plot analysis (a) Ramachandran plot of 1REV (b) Redocked complex AD4

*Most favourable region is displayed in red and amino acid residues are displayed in black squared

1REV revealed an 87.6% favourable region with 713 out of 937 amino acids before docking. However, in the redocked complex, it demonstrated 88.6%. Consequently, the percentage of most favourable regions showed relatively similar percentages before and after redocking in both receptors therefore, can conclude there wasn't any detriment to the receptor during the docking.

DISCUSSION

Protein-ligand docking is a procedure to find an optimum binding pocket between ligand and receptor. Through the formation of receptors, water molecules were eliminated to clear the binding pocket to the ligand. In addition, polar

hydrogens and hetero atoms were added and removed to form desirable hydrogen bonds by merging non-polar. Because these hydrogen bonds are essential to stabilize the protein-ligand docking (Lemmon and Meiler, 2013). While non-polar atoms were merged, it prevents unnecessary hydrogen bond formation and helps to elevate the protein structure by distributing their charge to the nearby carbon. The AD4 type was assigned using AutoDock to identify the hydrogen bond acceptors/donors and aliphatic/aromatic carbon and hydrogen bonding state of hetero atoms in BD; additionally, the SSD AD4 type was not added due to their PDBQT file containing assigned charges to form an optimised structure. Kollman charges were also added to both receptors (PDB ID: 1REV and 2R5Q), therefore able to calculate the template value of each amino acid residue of the receptor (Forli et al., 2016). Furthermore, the vina is a docking program based on a simple scoring function and rapid gradient optimization conformational search, whereas AutoDock is based on an empirical free energy force field and rapid Lamarckian algorithm search approach (Forli et al., 2016). The scoring function is immensely beneficial for structure-based drug development due to its quick and precise scoring, ability to detect an appropriate docking pose, and strong binding between a small molecule and its native receptor (Zheng et al., 2022). Additionally, the estimation of pair-wise atomic incorporates assessments for a variety of secondary interactions, including hydrogen bonding, electrostatics, dispersion/repulsion, and desolvation. With AD4, users can utilize empirical charge calculations using Kollman and Gasteiger charges. However, Empirical approaches for electrostatic potential calculation have the benefit of speed, but they also have certain disadvantages, therefore empirical scoring function yield less accuracy of binding affinity and geometry than the semi-empirical method (PM6 method). Furthermore, the Gasteiger charge calculation is unable to control the electrons, which provides a significant defect for the docking calculation of metalloproteins (Morris and Corte, 2021).

Through the preparation of ligands, the TORSDOF was configured to indicate the number of rotatable bonds allocated during BD that is allowed to rotate freely, and the torsion tree was used to enable the ligand to have multiple confirmations while binding to the protein. For SSD, docking was carried via fixed TORSDOF that was integrated into the ligand. The Gasteiger approach, which forms the basis of its orbital electronegativity partial equalisation, can be used to calculate partially charged proteins and ligands. But docking accuracy can be increased by polarizing the ligand using quantum mechanical methods like the AMBER score (Bikadi and Hazai, 2009). Moreover, GA uses the concept of natural genetics and biological evolution. The ligand has specified parameters (translation, orientation and confirmation) concerning the protein. In the GA, these are described as state variables, and each state variable corresponds to a gene. Whereas the ligand's state is generated by the genotype, the phenotype originates from the atomic coordinates (Phillips et al., 2018). In contrast, higher binding affinity (more negative score) and stability of a docked complex, which is obtained by the formation of lower potential energy are reflected in lower BA. Additionally, a high interaction number between HI and HPI was found to be associated with stability, BA, and exposure to bond breakage. Lower Ki is correlated with stronger drug potency and better binding energy; Ki is the lowest concentration required to suppress an enzyme by 50% or the percentage of a drug's binding efficiency (Meng et al., 2011) (Kataria and Khatkar, 2019). Furthermore, LE of lupatic acid and

lupeol (LE ≥ - 0.3) contribute to removing size effects and optimising compounds based on their effective binding and pharmacokinetic properties (García-Sosa, 2011). Moreover, chemicals are more likely to have an affinity for several targets whether their logP is too high. The term "ligand-lipophilicity efficiency" (LLE) was implemented to facilitate affinity optimization concerning logP. Furthermore, molecular size and lipophilicity are crucial aspects to consider to achieve optimum ADMET characteristics (Schultes et al., 2010).

Table 7:Comparison of best BE of the phytochemicals*my best(-11.15) their best(-10.11)

Receptor	Ligand	This study		Previous study		References
		BA (kcalmol^{-1})	Ki (nM)	BA (kcalmol^{-1})	Ki (nM)	
1REV	Lupeol	-11.15	6.67	-7.54	2970	(Singh *et al*, 2019)
	Carnsol	-9.74	72.61	-8.71	409.19	
	Carindinol	-9.72	76.62	-10.11	39.14	
	Gallic acid	-9.03	239.98	-6.58	14940	
	Carindone	-8.39	708.28	-8.85	324.25	
	Toddanone	-7.04	6920	-6.02	-	(Priya *et al*., 2015)

In this study, lupeol was established to be the most efficient phytochemical. Furthermore, its BE and Ki values were elucidated to significantly higher performance than in earlier research.

Table 8: The common amino acid interactions and H-bonds of best docking ligands using SSD Of 1REV

Receptor	Software	Type of the ligand	HPIs (This study)	Previous study (Singh *et al*.,2019)
				Active amino acids residues
1REV	AD4	FDA-approved drugs	LEU100, VAL179, VAL106, TRP229, TYR181	-
		Phytochemicals	LEU100, PHE227, VAL106, LEU234, TYR188, TYR318, TRP229, VAL179, TYR181, TRP266	LEU100, VAL106, VAL179, ILE180, TYR180, TYR181, PHE227, LEU234, TYR318

These LEU100, VAL106, VAL179, TYR181, PHE227, LEU234 and TYR318 interactions were found respectively in the previous and this study. PyMOL was used to superimpose and validate the redocked AD4 pose, and to obtain good performance, the redocked complex's binding affinity needed to be lower and its RMSD value needed to be < 2.00Å (García-Sosa, 2011). Therefore, the docking methodology was validated by obtaining along with confirmation of ligand orientation. However, there are some limitations in AD4 such as the presence of lots of degrees of freedom and docking methods are not accessible to confirmational space, the protein targets often show significant conformational flexibility. However, AD4 and vina employ several simplifications that affect the results obtained. The main simplification is the use of rigid receptors because it can reduce the size of conformation space and scoring function. These challenges can be avoided by using receptor structure that has already been docked complexes, docking to a variety of distinct receptor structures, and using explicit receptor side chain flexibility during docking (Forli et al., 2016).

CONCLUSION

In conclusion, a total of 27 phytochemicals were used for protein-ligand docking and among all the phytochemicals withaferin A exhibited the most potent inhibitor against 1REV with the lowest binding energy. Furthermore, efavirenz was the best FDA-approved drug against 1REV. In addition, LYS101, lLYS103 and ILE180 were identified as amino acids that interacted with HIs in 1REV respectively. However, 1REV demonstrated the best performance among those ligand-binding receptors which was linked with HPIs in LEU100 and VAL106. According to the analysis of ADMET properties, the best phytochemicals for oral bioavailability were withaferin. A and lupeol respectively. As shown by the findings, withaferin A and lupeol were the most effective phytochemicals because of their non-toxicity, accepted Lipinski rules and excellent GI absorption due to optimum lopP. Finally, all of the study's objectives were accomplished, and further work will be done in the future.

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