# Virtual Screening of Phytochemical Compounds as Potential Inhibitors against HIV-1 Resistance to protease inhibitor

Thirunavukkarasu Umaarasu[1] , Kesavaram Padmavathy[1*], Dharmalingam Thirunavukkarasu[2], Shanmugam Gnanendra [3*]

[1]*Department of Microbiology, Research Laboratory for Oral & Systemic Health, Sree Balaji Dental College & Hospital, BIHER, Chennai. 600100, Tamil Nadu, India.*
[2]*Department of Microbiology, Government Mohan Kumaramangalam Medical College, Salem, Tamil Nadu, India.*
[3]*Department of Biotechnology, College of Life and Applied Sciences, Yeungnam University, Gyeongsan, Gyeongbuk, 38541, Korea*

**Abstract**

Despite the availability of anti-retroviral agents, Human Immunodeficiency Virus (HIV) infection continues to be a significant global public health issue with a higher rate of morbidity and mortality. The HIV-1 protease is an aspartyl protease that is required for proteolytic processing of the gag and gag-pol polyprotein precursors and is indispensable for proper virion assembly and maturation. The rapid emergence and dissemination of drug-resistant HIV-1 variants and the adverse side effects of currently used HIV-1 protease inhibitors (PIs) remain critical factors that necessitate the discovery of newer phytocompounds with potential antiviral activity against HIV-1. The study was proposed to evaluate the binding efficiency of the phytochemical compounds from the methanolic extracts of *Ricinus communis, Andrographis paniculata* and *Withania somnifera* against the HIV-1 protease, mutant and wild-type and to compare with the standard PI, nelfinavir. The 3D structures of the HIV-1 protease with mutations *viz.*, V32I, I47V, and V82I were developed by homology modeling. A total of 120 phytochemical compounds from the *Ricinus communis, Andrographis paniculata*, and *Withania somnifera* were virtually screened against the binding site of HIV-1 protease, wild-type, and mutant type. The docking interactions of APC-4 with the HIV-1 protease mutant type exhibited a binding affinity of -27.1425 kJ/mol while WSC-31 with HIV-1 protease wildtype exhibited -25.4546 kJ/mol, better than the binding affinity of the conventional protease inhibitor, Nelfinavir. These phytochemical compounds, APC-4 and WSC-31 could be potential alternatives to the conventional PI, Nelfinavir against HIV-1.

**Keywords:** HIV-1 Protease; Phytochemicals; Nelfinavir; docking; virtual screening

## 1. INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) caused by the retrovirus Human Immunodeficiency Virus (HIV) is one of the most important infectious diseases with high mortality in many developed and developing countries. According to current data from WHO (2017), it is estimated that there are 36.9 million people are living with HIV/AIDS worldwide. Majority of the global burden of HIV infections is reported in Africa with approximately 25.7 million people living with HIV, followed by 3.5 million in South-east Asia [1]. The global coverage of antiretroviral therapy (ART) among people living with HIV is estimated to be 59% (2017) and hence, the HIV related deaths have declined from 1.5 million in 2000 to 0.9 million in 2017. Also, there is a reduction in the number of people newly infected with HIV from 2.8 million in 2000 to 1.8 million in 2017 [2].

Human Immunodeficiency Virus (HIV-1) infection is universally considered as a chronic disease that slowly progresses to Acquired Immunodeficiency Syndrome (AIDS). HIV has a high genetic diversity due to the fast replication cycle of the virus coupled with the high error prone rate of its RT enzyme [3]. The HIV polyprotein precursor is encoded by relatively simple genomes consisting of gag, pol and env open reading frames. The *gag* gene encodes the structural capsid, nucleocapsid, and matrix protein; *env* undergoes multiple alternative splicing events to regulatory protein; while, *pol* encodes essential viral enzymes necessary for viral replication [4]. The HIV-1 protease (HIV-1 PR) is an aspartyl protease that is required for proteolytic processing of the gag and gag-pol polyprotein precursors to yield the viral enzyme and structural proteins and is indispensable for proper virion assembly and maturation. HIV-1 PR contains a homodimeric C-2 symmetric structure, and each monomer contributes one catalytic aspartic residue along with threonine and glycine residues which are flexible and a flap that favors the binding of substrate and inhibitors [5].

In the absence of an effective vaccine, drugs remain the only therapeutic tool for the treatment of HIV-1 infections. Unfortunately, ART once initiated, need to be continued lifelong. This places a special burden on the design of anti-HIV drugs. The protease inhibitors (PIs) and reverse-transcriptase inhibitors have resulted in the unprecedented success of HIV/AIDS chemotherapy. However, owing to the rapid emergence of drug-resistant HIV-1 variants and transmission of these resistant viral strains along with the adverse side effects of the currently used HIV-1 PIs, ART remains a clinical challenge [6]. The FDA approved HIV-1 protease inhibitors including atazanavir, indinavir, nelfinavir and saquinavir for HIV treatment are very peptide-like and have poor bio-availability [7].

To overcome these issues, there is a need for the development of new PIs with improved activity against drug-resistant variants and excellent pharmacokinetic and safety profiles. The pharmaco-informatics approaches including virtual screening and molecular docking have become pivotal techniques in the pharmaceutical industry

for lead discovery. Hence in the present study binding efficiencies of the phytochemical compounds from selected medicinal plants were evaluated against the HIV protease receptor from mutant and wild-type regarding molecular docking and compared with the standard protease inhibitor nelfinavir.

## 2. METHODOLOGY

### 2.1. HIV protease wild-type and mutant structures

Crystal Structure of HIV-1 Subtype C Protease complexed with Nelfinavir (PDB ID: 2R5Q), an aspartyl protease that is required for proteolytic processing of the gag and gag-pol polyprotein precursors to yield the viral enzyme and structural proteins and is indispensable for proper virion assembly and maturation was retrieved from PDB database [8]. The 3D structure of the HIV-1 protease with mutations such as V32I, I47V, and V82I was developed by using the atomic coordinate file of 2R5Q as template and the model was evaluated.

### 2.2. Homology Modeling and model evaluation

A total of five 3D models of the HIV-1 PR sequences with 32I, 47V, and 82I mutations were built from the starting structure of the template (2R5Q) by satisfying the spatial restraints through random generation [11]. Among the generated 5 model, the model with least RMSD value was used for further analysis after minimizing its energy by using GROMOS [12]. Also, the stereochemical parameters of the energy-minimized model were considered to evaluate the quality of the generated models. The phi and psi angles representing the stereochemical parameters of the model was generated by using PROCHECK [13], at SAVES structural analysis server [14].

### 2.3. Binding Pocket Prediction

From the Crystal Structure of HIV-1 Subtype C Protease complexed with Nelfinavir, the binding pocket in the modeled HIV-1 PR mutant was identified by superimposing the template and target protein structures. Eventually, the predicted binding site was used to explore the binding affinities of phytochemical compounds from medicinal plants including *Ricinus communis* (leaf), *Andrographis paniculata* (leaf) and *Withania somnifera* (root) through virtual screening [15].

### 2.4. Lead compounds

The 2D structure of the phytochemical compounds of *Andrographis paniculata* (APC), *Ricinus communis* (RCC) , and *Withania somnifera* (WSC) were drawn in ACD-Chemsketch [16], and their SMILES notation was obtained. Further, these 2D structure were converted into 3D SDF files by using 'Online SMILES convertor and Structure file generator' [17] for docking studies. All the compounds were optimized and protonated prior to docking.

### 2.5. Molecular Docking

The 3D structure of phytochemical compounds in SDF format were virtually screened to reveal their binding efficiencies through docking in the predicted binding pockets of modeled HIV-1 protease mutant and crystal structure of HIV-1 PR (wild-type) by using FlexX [18] with the default docking parameters such as triangle matching base placements, zero full score and No score contributions and threshold for full score and no score

contributions of 30 & 70 respectively, Clash handling values of 2.9 Å and 0.6 for protein ligand clashes with maximum allowed overlap volume and intra-ligand clash factors while considering the hydrogen in internal clash tests and 200 as the default docking values for maximum number of solutions per iteration and also per fragmentations.

Further, the docking protocol was validated with active compound (Nelfinavir) and inactive compounds (chloramphenicol). The docking interaction was explored with a conventional protease inhibitor, nelfinavir and inactive compound (chloramphenicol) against both mutant and wildtype.

### 2.6. Docking Interactions

The docking interactions that envisage the binding affinities of the phytochemical compounds within the predicted binding pockets of both HIV-1 mutant and wild-type protease were analyzed by using pose-view module of LeadIT [19]. The Hbond and non-bond interactions between ligand (phytochemicals) and receptor (protein binding pocket amino acids) are clearly visualized.

## 3. RESULTS AND DISCUSSION

HIV drug resistance is one of the major hurdles for achieving and maintaining successful viral suppression. Most data on the genetic mechanisms of HIV-1 drug resistance are from studies of subtype B viruses, the predominant subtype in the North American and Europe. Several of studies suggest that the currently available PR (Protease) and RT (Reverse Transcriptase) inhibitors are as active against non-B viruses as they are against subtype B viruses [20-24].

In this study, the potential HIV-1 protease ligand nelfinavir interactions with both mutant and wildtype HIV-1 Subtype C Protease were analyzed by using the ligand-receptor interactions through molecular docking. Molecular docking is one of the best filtering methods and a crucial technique in the drug design process. The Molecular Docking protocol of the FlexX was used to dock the retrieved compounds by virtual screening. The protocol first analyses the provided cavity and then selects the region of the protein as the active site, and secondly dock the ligands to the chosen site. 3D regular grids of points are employed for site detection and also for estimating the interaction energy of the ligand with the protein during docking. The protein receptor of the HIV protease was selected from Protein Data Bank (RCSB-PDB) for the molecular docking study. The 3D structure of the HIV-1 Subtype C Protease complexed with Nelfinavir is shown in Figure. 1.

### 3.1. Homology modeling and validations

To explore the effect of the mutation on the drug binding, the HIV-1 protease protein sequences with V32I, I47V, and V82I mutations was used to build the 3D structure. The homology model of was built by using the atomic coordinate files of crystal structure of HIV-1 Subtype C Protease complexed with Nelfinavir retrieved from PDB (PDB ID: 2R5Q) with modeller9v9. The built 3D structure of the mutant HIV-1 protease was energy minimized (200 cycles of Steepest Descent) by using SwissPDB viewer with GROMOS 43B1 force field. The built model was

validated through Ramachandran plot by using the PROCHECK, Verify 3D and ERRAT plots at SAVES server. The Ramachandran plot of the energy-minimized model showed most of the residues in the most favorable region (94.9%), additionally allowed regions (5.1%) and 0.0 % in the generously allowed and disallowed regions. Also, the model has PASS value of 86.36 from Verify 3D and ERRAT Plot showing the generated model as good

high resolution as the regions of the modelled structure that can be rejected at the 95% and 99% of confidence is very low with a stretch of only 7 amino acids (Supplementary Figure S1 and Supplementary Table T1). Thus the model was considered best as it exhibited a number of residues in the most favorable regions and also the low number of residues in disallowed region.
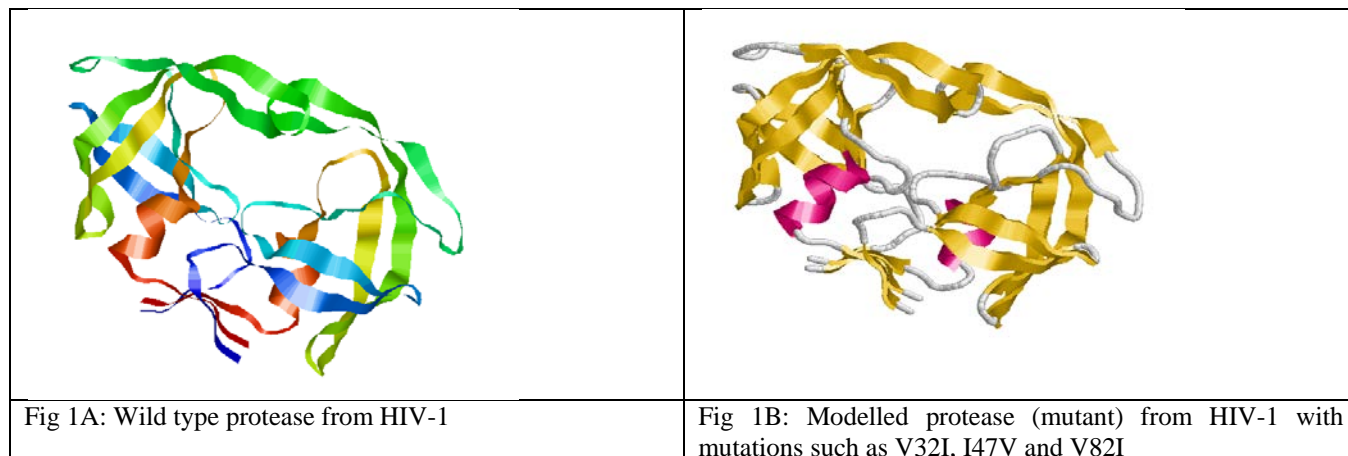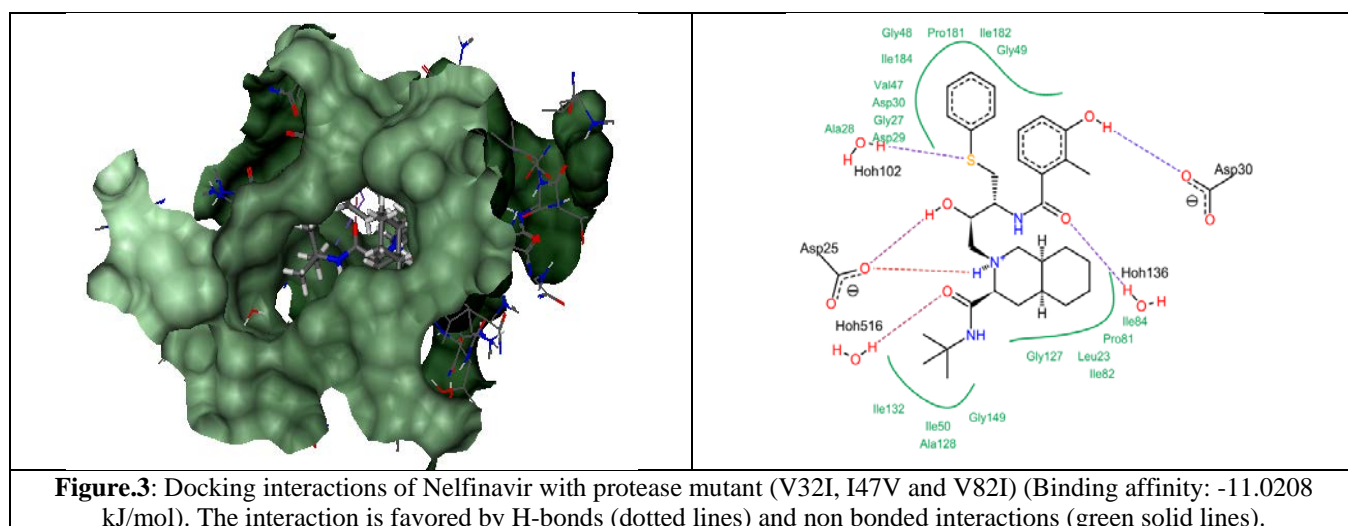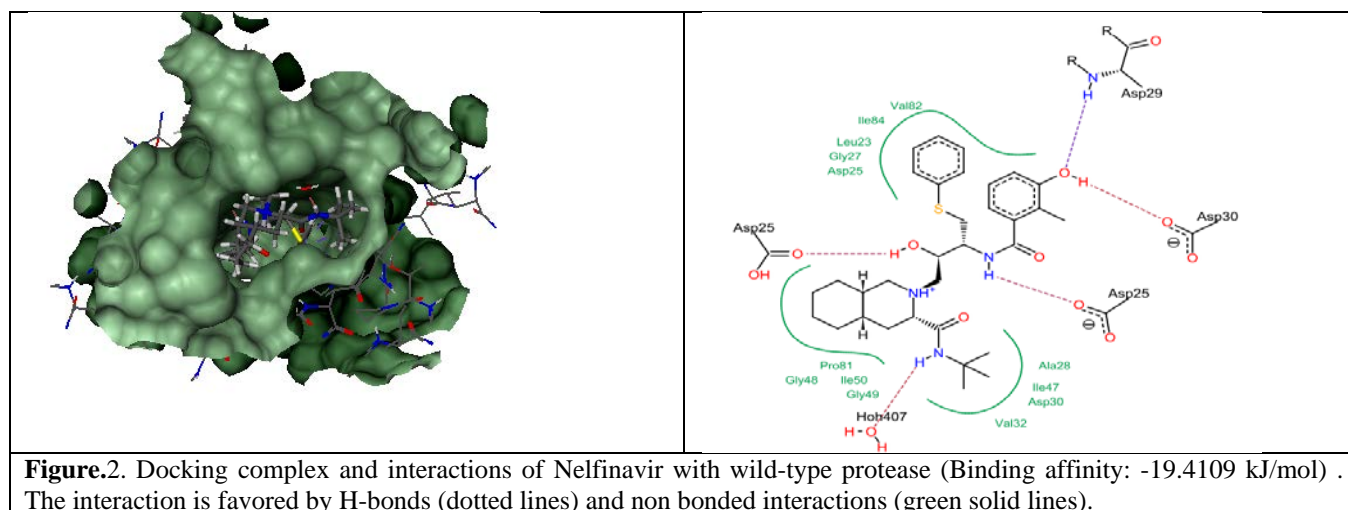


| Fig 1A: Wild type protease from HIV-1 | Fig 1B: Modelled protease (mutant) from HIV-1 with mutations such as V32I, I47V and V82I |

**Figure.**1. The 3D structure of wild type (1A) and modelled protease (mutant) (1B) from HIV-1.



**Figure.**2. Docking complex and interactions of Nelfinavir with wild-type protease (Binding affinity: -19.4109 kJ/mol) . The interaction is favored by H-bonds (dotted lines) and non bonded interactions (green solid lines).



**Figure.**3: Docking interactions of Nelfinavir with protease mutant (V32I, I47V and V82I) (Binding affinity: -11.0208 kJ/mol). The interaction is favored by H-bonds (dotted lines) and non bonded interactions (green solid lines).

### 3.2. Docking Studies

The docking interactions of phytochemical compounds (Supplementary Figure S1) from *Andrographis paniculata* (APC), *Ricinus communis* (RCC), and *Withania somnifera* (WSC) were used to determine their inhibition activity against the crystal structure of HIV-1 Subtype C Protease (wild-type) and mutant protease through docking studies.

### 3.3. Docking interactions of Nelfinavir against crystal structure of HIV-1 Subtype C Protease (Wildtype)

The docking interactions of Nelfinavir against the wildtype HIV-1 exhibited the binding affinity of -19.4109 kJ/mol. This interaction is favored by the formation of H bond and non-bonded interactions. The H- bonds are supported by the amino acids such as Asp29, Asp30, and Asp25 within the active site. The docking complex and binding interactions of Nelfinavir with wildtype HIV-1 protease are given in Figure.2.

### 3.4. Docking interactions of Nelfinavir against the built HIV-1 protease with mutations (Mutant)

The docking interactions of Nelfinavir against the mutant HIV-1 exhibited the binding affinity of -11.0208 kJ/mol. This interaction is favored by the formation of the H bond and non- bonded interactions. The h bonds are supported by the amino acids, Asp30, and Asp25 with the active site of mutant protease. The mutation at V32I, I47V, and V82I might have the significant role in imposing the low binding affinity. Interestingly, it is observed that the H bond formation with Asp29 is absent in the mutant when compared to wildtype. The docking complex and binding interactions of Nelfinavir with mutant HIV-1 protease are given in Figure.3. The comparison in the binding affinities of nelfinavir against the wild-type and mutant protease is presented in Table.1. In comparison, the wild type protease exhibited the docking interactions with Asp29, which is not observed in mutant. Also, the mutant exhibited the docking interactions with three while molecules, while the wildtype exhibited the interaction with only one water molecules. This difference in binding interactions and binding affinities reveals that these mutations affect the binding nelfinavir.

**Table.1.** Comparison of binding affinities of Nelfinavir against wild-type and mutant HIV-1 protease.

| Protein | Wild type/ mutant | Docking score (kJ/mol) |
|---|---|---|
| Protease | No mutation (Wild type) | -19.4109 |
| | V32I, I47V and V82I (mutant) | -11.0208 |

### 3.5. Virtual screening of phytochemical compounds against crystal structure of HIV-1 Subtype C Protease (Wildtype)

A total of 133 phytochemical compounds from the *Ricinus communis (RCC)* (33 compounds), *Andrographis paniculata (APC)* (34 compounds) and *Withania somnifera (WSC)* (66 compounds) were virtually screened against the binding site of crystal structure of HIV-1 Subtype C Protease (wild-type). Theoretically, it is observed that most of the compounds exhibited better docking interactions in terms binding affiniti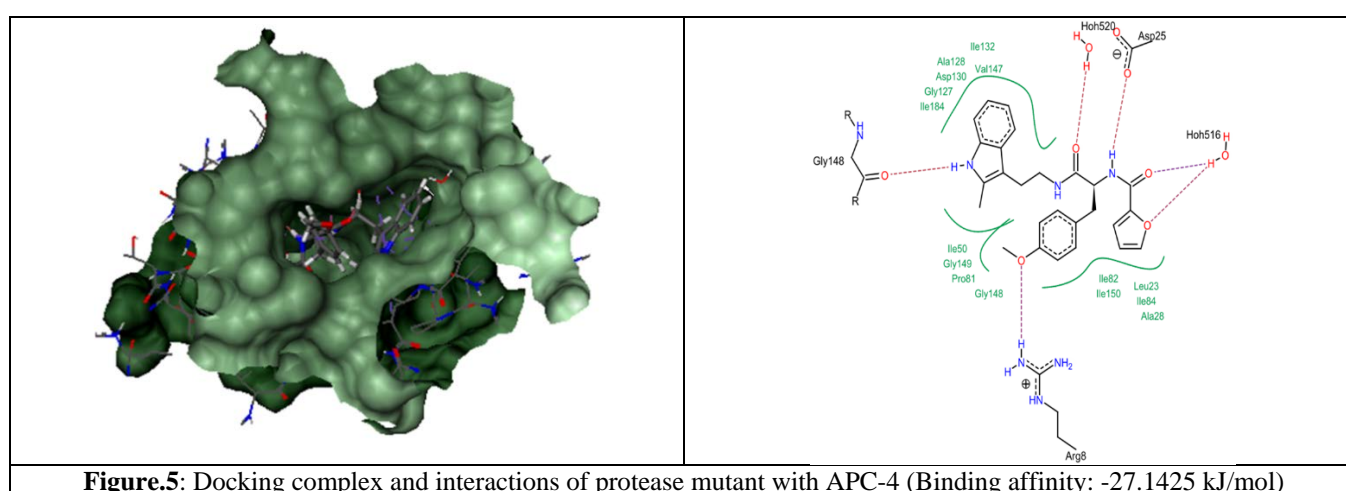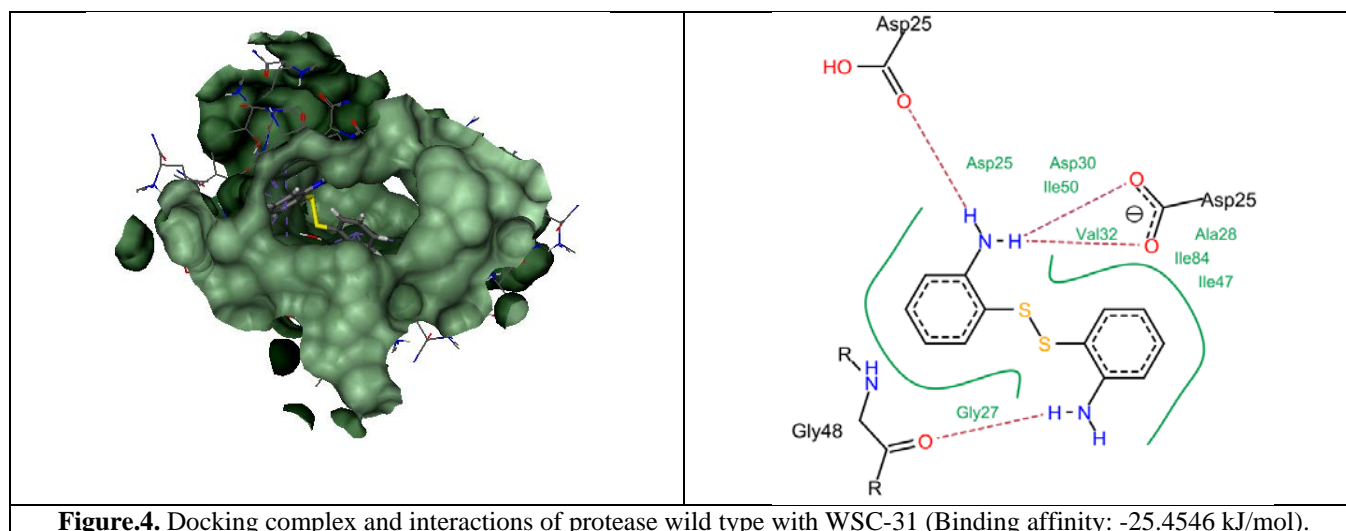es (kJ/mol). Among the best-docked compounds, the compound (WSC-31) (2-[(2-aminophenyl)disulfanyl]aniline) from *Withania somnifera* showed the highest binding affinity (-25.4546 kJ/mol) when compared to the conventional protease inhibitor, nelfinavir (-19.4109 kJ/mol). Also, the compounds that exhibited better docking score higher than that of nelfinavir were found from *Andrographis paniculata*. In line with this, the best five compounds that exhibited higher docking score in comparision with nelfinavir are shown in table 2. The binding affinities of all the compounds against crystal structure of HIV-1 Subtype C Protease (Wildtype) are given in supplementary table (Table S2). The docking interactions of WSC-31 (2-[(2-aminophenyl)disulfanyl]aniline) against the wildtype HIV-1 is favored by the formation of H bond and non-bonded interactions. The H bonds are supported by the amino acids such as Asp25 and Gly48 with the active site of wild-type protease (Figure.4).

**Table.2.** The binding interactions of best five phytochemical against mutant and wild type HIV-1 protease.

| Phytochemicals | Protease mutant | Phytochemicals | Protease wild type |
|---|---|---|---|
| APC-4 | -27.1425 | WSC-31 | -25.4546 |
| WSC-33 | -26.2197 | APC-5 | -24.2563 |
| APC-5 | -23.2949 | WSC-39 | -23.6818 |
| WSC-26 | -22.133 | APC-4 | -21.3861 |
| WSC-31 | -21.5536 | WSC-33 | -20.4196 |

### 3.6. Docking interactions of phytochemical compounds against HIV-1 protease with mutations (Mutant)

All the 133 phytochemical compounds from the *Ricinus communis (RCC)* (33 compounds), *Andrographis paniculata (APC)* (34 compounds) and *Withania somnifera (WSC)* (66 compounds) were virtually screened against the binding site of HIV-1 protease with mutations (mutant). Theoretically, it is observed that most of the chemical compounds exhibited better docking interactions with better binding affinities in terms of kJ/mol. Among the best-docked compounds, the compound (APC-4) (2S)-2-[(furan-2-yl)formamido]-3-(4-methoxyphenyl)-N-[2 -(2-methyl-1H-indol-3-yl)ethyl]propanamide from *Andrographis paniculata* has shown the highest binding affinity when compared to the conventional protease inhibitor, nelfinavir. Also, the best five compounds with docking scores greater than nelfinavir are from *Andrographis paniculata* and *Withania somnifera* (Table 2). The binding affinities of all the compounds against protease (mutant) are given in the supplementary table. The docking interactions of APC-4 against the mutant HIV-1 exhibited the binding affinity of -27.1425 kJ/mol. This interaction is favored by the formation of H bond and non-bonded interactions. The h bonds are supported by the amino acids such as Arg8, Asp25, and Gly148 with the active site of mutant protease. The docking complex and binding interactions of APC-4 with mutant HIV-1 protease are given in Figure.5.

**Figure.4.** Docking complex and interactions of protease wild type with WSC-31 (Binding affinity: -25.4546 kJ/mol).



**Figure.5**: Docking complex and interactions of protease mutant with APC-4 (Binding affinity: -27.1425 kJ/mol)

Ungwitayatorn et al. [25] reported the docking interactions of non-peptide HIV-1 protease (HIV-1 PR) inhibitors such as chromone derivatives. The chromone molecules form hydrogen bonding interaction with Asp25, Asp25, Ile50 and Ile50 and hydrophobic interaction with Val32, Ile50, Pro81, Val82, and Ile84. These docking studies implied that the conserved amino acid Aspartic acid and Glycine (Gly26) in the catalytic site of HIV-1 Protease receptor are crucial in the binding of anti-HIV-1 Protease inhibitors. These docking interactions imply that the NH group and =O present in the compounds favors the h bond interactions. Hence these findings throw light for the design of novel anti-HIV-1 protease inhibitors and also envisages that the amino acids Aspartic acid (Asp 25 and 29) and Glycine (Gly148) should be considered during its design for implying its action as a best anti-HIV-1 Protease compound against the potential target of HIV-1 Protease.

Thus, considering the binding affinities of the phytochemical compounds from *Andrographis paniculata* and *Withania somnifera,* it is envisaged that these compounds might possess anti-viral activities against the HIV-1 protease. Also, these docking studies suggested that these phytochemical compounds could be employed in the treatment of HIV-1 as an alternative to the conventionally used Nelfinavir. Interestingly, it is observed that the binding affinity of Nelfinavir has decreased in mutant protease, while the binding affinities of the phytochemical compounds have shown higher affinities both in mutant and wild-type proteases. Thus this study, significantly suggest that this compound might lead to the design of novel PI inhibitors against the drug-resistant HIV-1 viruses.

## 4. CONCLUSION

The 3D structure of HIV-1 protease with mutations V32I, I47V and V82I, were developed through homology modeling with reference to wild-type protease as the template (PDB ID:2R5Q) and validated. Further, the effect of mutations on the drug resistance was determined through docking studies. The conventionally used protease inhibitor, Nelfinavir was docked against both wild-type and mutant protease. Also, the anti-retroviral activity of the phytochemical compounds from *Andrographis paniculata*, *Ricinus communis,* and *Withania somnifera* was evaluated. It is observed that nelfinavir exhibited -19.4109 kJ/mol and -11.0208 kJ/mol against HIV-1 protease wild-type and mutant respectively. Interestingly, the binding affinities of the phytochemical compounds exhibited better score when compared to that of standard protease inhibitor. The

docking interactions of compound APC-4 exhibited -27.1425 kJ/mol and WSC-31 exhibited -25.4546 kJ/mol against HIV-1 protease mutant and wildtype respectively. Thus, this docking study suggests that these phytochemical compounds could be employed in the design of alternative HIV-1 therapeutics as in place of conventionally used Nelfinavir.

**Conflicts of Interest:** The authors declare no conflict of interest.

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