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In Silico Phytochemical Screening for Dengue Virus

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Abstract

By and by, the world is fighting with dengue for long decades. However, the pathogenesis of dengue has not yet been fully elucidated. Dengue fever is a virus infection that is spread by the Aedes aegypti mosquito and can cause severe disease especially in children. Dengue fever is a major problem throughout the world especially tropical and sub-tropical regions of the world.The disease is still tearing down the world. Millions of researchers are still continuing for the potential medication up-and-comer that could help the medical care framework to keep world health. We present a docking-based screening using a quantum mechanical scoring of a library built from approved drugs and compounds that Gingerol, Honokiol, Thiosulfinate, Saponin, Coumarin, Apigenin, Limonin, Baicalin, Quercetin, Tannin amine, Ajoene, Curcumin, with proteins with PDB id's 4OIG, 6VSO could display antiviral activity against destructive dengue virus. Clearly, these compounds should be further evaluated in experimental assays and clinical trials to confirm their actual activity against the disease. We hope that these findings may contribute to the rational drug design against Dengue virus.

Introduction

Dengue has become a serious public issue over the globe since the beginning. The earliest descriptions of an outbreak of dengue date from 1779. Although an estimated 100-400 million infections occur each year throughout the world. It is spread by *Aedes aegypti* mosquito It affects individuals worldwide especially in tropical regions of the world and even though many remedies are discovered there is no proper immunization yet for these infections. Which is viewed as a major threat to general wellbeing of the world? There is a pressing need to create an intense enemy of these diseases, specialists for the avoidance of the flare-up and stop viral contaminations. Repurposing of realized little particles is by all accounts an exceptionally productive path so as to create strong medications to battle diseases in this brief timeframe. As of late, various endeavors were made to plan novel inhibitors or utilize drug repurposing ways to deal with recognition hostile to medications.

Procedure:

1. Ligand Screening:

For the initial Ligand screening purposes, a web-based tool named SwissADME (https ://www.swiss adme.ch/) was used to eliminate a few compounds according to Lipinski's rule of five parameters. For a compound to qualify as ligand it should Have < 500 Da molecular weight, a high lipophilicity i.e. value of Log P being less than 5, hydrogen bond acceptors being less than 10 and H-bond donors less than 5. Any compound with more than 2 violations was ruled out for further study (Lipinski2004).

2. Protein Preparation and Active site Determination:

Required protein in PDB format was downloaded from the website **rcsb.org**, commonly known as the **Protein Data Bank**. 3D conformers of the ligand were downloaded from PubChem.
Using **PyMOL (Version 2.4.1)** software water molecules as well as native ligands from the protein were removed, defined as cleaning/purification of the protein for further application. **Using a web server called Deep Site** Active Pockets of the proteins was calculated. The results calculated by the web server were in the form of different ids, centers and scores.
Scoring In deep site was using neural networking based on following instructions using DCNN architecture.
https://academic.oup.com/bioinformatics/article/33/19/3036/3859178 Center values for the grid were selected keeping score greater than 0.98.

UCSF Chimera (Version 1.14) was used to prepare the receptor using DockPrep function. **Dock Prep** prepared structures for Docking using these functions:

- Deleting water molecules
- Repairing truncated sidechains
- Adding hydrogens
- Assigning partial charges
- Writing files in Mol2 format

1. In silico Docking Using Auto dock Vina Auto dock Vina (Version 1.1.2) along with UCSF Chimera (Version 1.14) was used for molecular **Docking Studies**. Center values and size of the grid of different scores were used from **DEEPSITE** calculations done above.

Following Parameters were set in auto dock vina.

Receptor options –

1. **Add hydrogens in Chimera** (**true**/false) – whether to add hydrogens in Chimera before calling the script. The receptor prep script will check for hydrogens and add them if they are missing. AutoDock Vina needs the polar (potentially H-bonding) hydrogens to identify atom types for scoring purposes.
2. **Merge charges and remove non-polar hydrogens** (**true**/false) – note AutoDock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the processed receptor
3. **Merge charges and remove lone pairs** (**true**/false) – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the processed receptor (and there may not have been any lone pairs to start with)
4. **Ignore waters** (**true**/false)
5. **Ignore chains of non-standard residues** (**true**/false) – ignore chains composed entirely of residues other than the 20 standard amino acids.
6. **Ignore all non-standard residues** (true/**false**) – ignore all residues other than the 20 standard amino acids.

For Ligands

- **Merge charges and remove non-polar hydrogens** (**true**/false) – note Auto Dock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the ligand output files
- **Merge charges and remove lone pairs** (**true**/false) – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the ligand output files (and there may not have been any lone pairs to start with)

Docking parameters

- **Number of binding modes** (1-10, **10**) – maximum number of binding modes to generate
- **Exhaustiveness of search** (1-8, **8**) – thoroughness of search, roughly proportional to time
- **Maximum energy difference (kcal/mol)** (1-3,**3**) – maximum score range; binding modes with scores not within this range of the best score will be discarded.

The docking results were calculated by Auto dock vina using it's Scoring function and results were displayed in the form of Scores and RMSD values. Docking results with the highest value score accompanied by negative sign and least RMSD values were chosen for further studies.

4. Residue Analysis

PyMOL was used for visualization of interactions of the docked structure at the ligand sites. **Discovery Studio 2020** was used to study the ligand interactions and total number of residues. It was also used to plot the 2D structure of the interactions and residues.

Statistical Analysis: Descriptive, estimation and Hypothesis testing with confidence interval of 95% was applied to data using formula 1 given belw.

$$CI = \bar{x} \pm z\frac{s}{\sqrt{n}}$$

CI = confidence interval
$\bar{x}$ = sample mean
z = confidence level value
s = sample standard deviation
n = sample size

Formula 1 used for calculation of confidence interval

Results and Discussion:

Molecular Docking:

The docking result was obtained from Auto dock vina in the form of Dock score for the two proteins docked with above mentioned ligands.

Dengue Protein Docking Results:

PDB-ID 4OIG

For 4OIG, two active sites were selected out of which the 0thactive site was selected with a Deep site score. Table 1. The selection was made on the basis of the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 1 and Table 2 shows the post statistical docking scores with Ligand Protein Interactions.

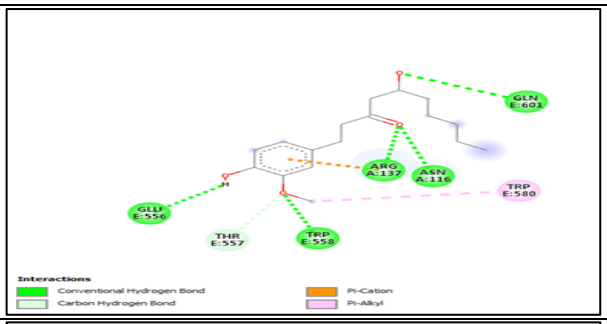
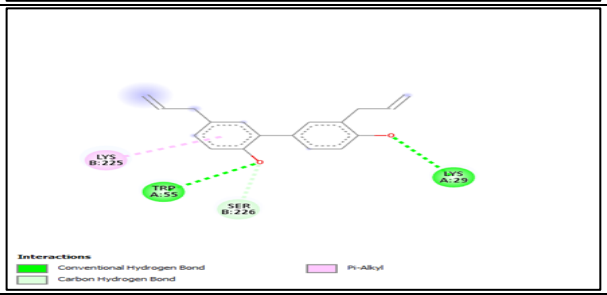
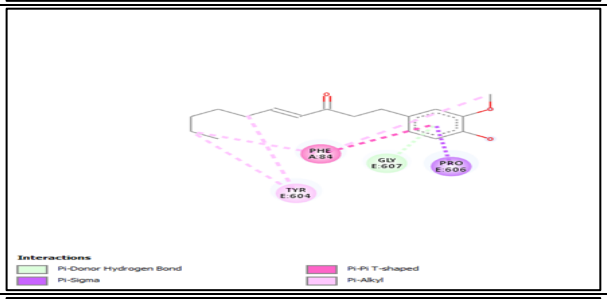
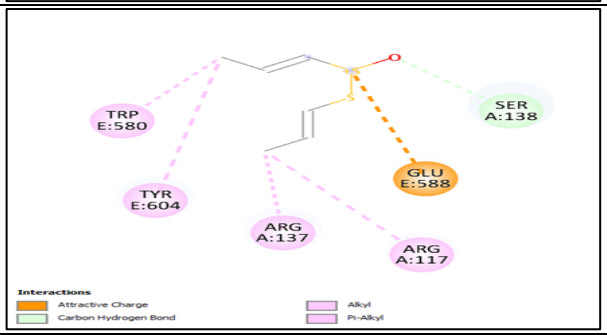
Table 1: shows the selected sites obtained from Deep Site based on DCNN algorithm

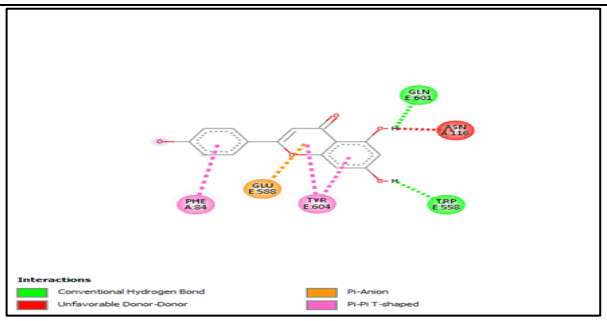
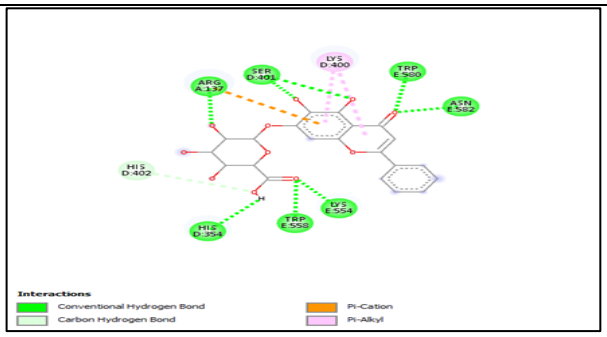
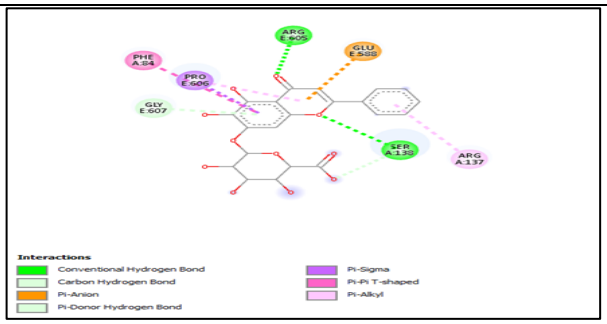
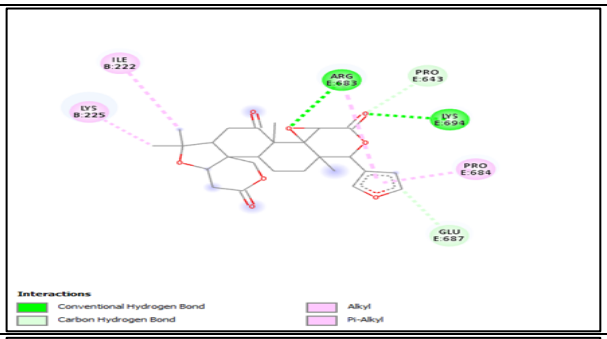
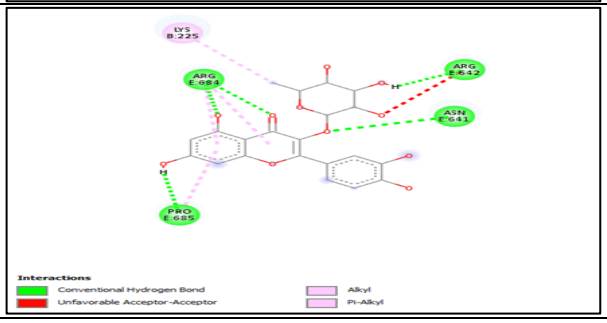
Site	Score	Selected/Not Selected
0		
1		

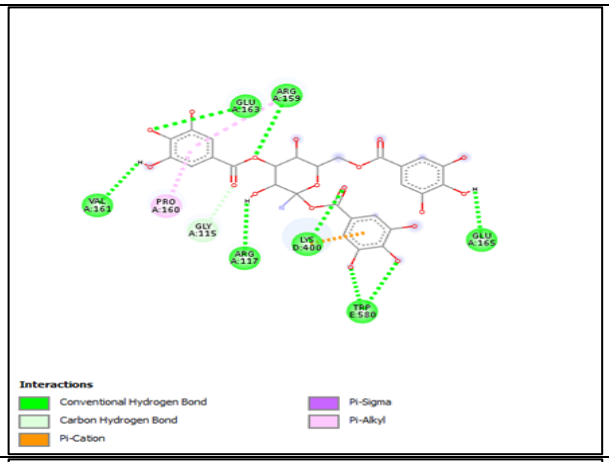
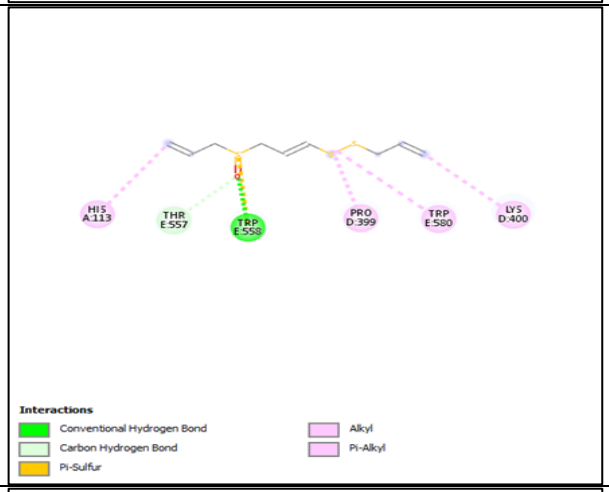
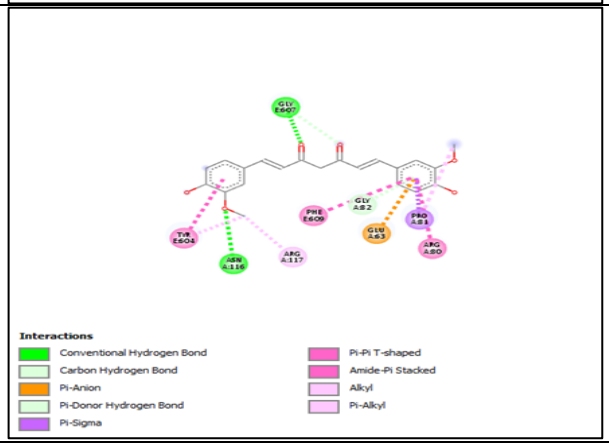
Ligands	Dockscore
Gingerol	-6
Honokiol	-6.2
Shogaol	-6.2
Thiosulfinate	-4.1
Apigenin	-8.2
Baicalin	-8.9
Coumarin	-8.8

Limonin	-8.7
Quercetin	-7.3
Tannin amine	-8.2
Ajoene	-4.1
Curcumin	-8.7

Table 2: 4OIG

Ligands	Dockscore	Interactions
Gingerol	-6	
Honokiol	-6.2	
Shogaol	-6.2	
Thiosulfinate	-4.1	

Apigenin	-8.2	
Baicalin	-8.9	
Coumarin	-8.8	
Limonin	-8.7	
Quercetin	-7.3	

Tannin amine	-8.2	
Ajoene	-4.1	
Curcumin	-8.7	

PDB-ID 6VSO

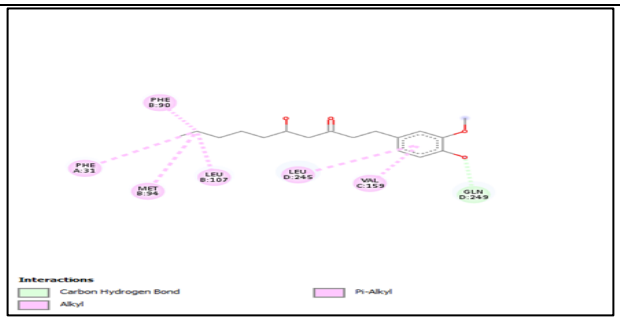
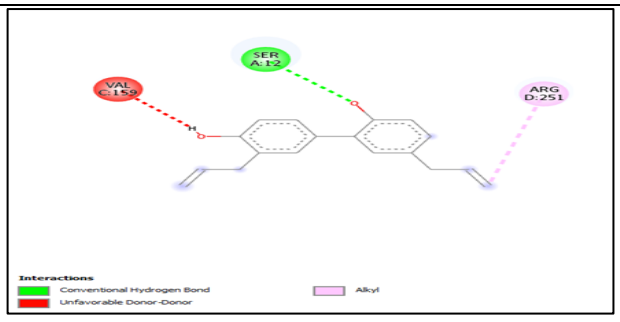
For 6VSO, 0th site,out of the two active sites was selected with a Deep site score of 0.985. The selection was made on the basis of the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 3 and Table 4 shows the post statistical docking scores with Ligand Protein Interactions.

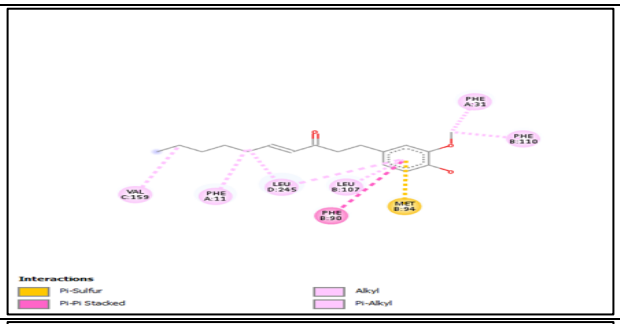
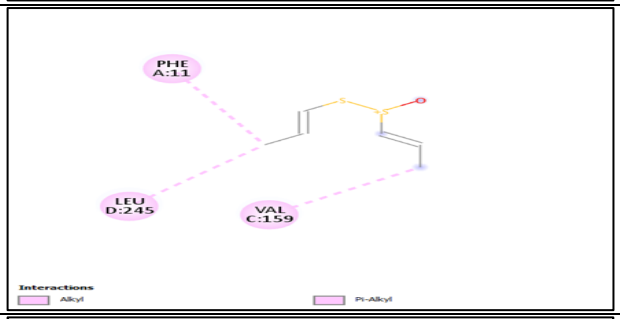
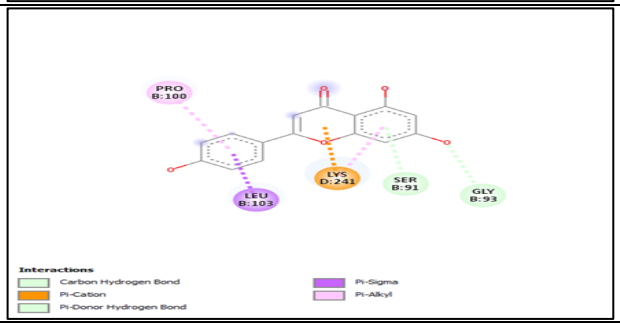
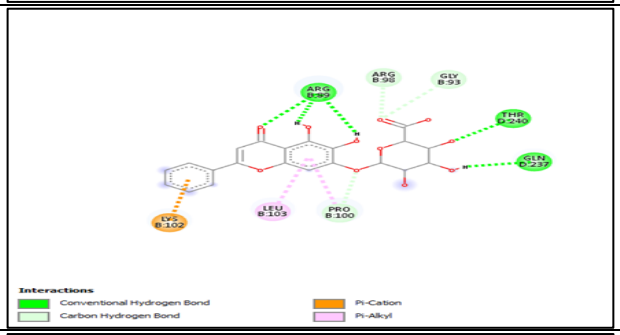
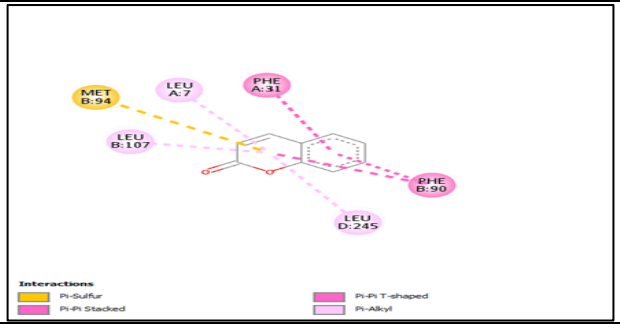
Table 3

Sites	Score	Selected /Not selected
0	0.985	
1	0.981	

Ligands	Dockscore
Gingerol	-6.7
Honokiol	-6.7
Shogaol	-7.1
Thiosulfinate	-4.4
Apigenin	-6.8
Baicalin	-7.8
Coumarin	-6.7
Limonin	-8.8
Quercetin	-6.9
Tannin amine	-7
Ajoene	-4.7
Curcumin	-7.8

Table 4 6VSO

Ligands	Dockscore	Interactions
Gingerol	-6.7	
Honokiol	-6.7	

Shogaol	-7.1	
Thiosulfinate	-4.4	
Apigenin	-6.8	
Baicalin	-7.8	
Coumarin	-6.7	

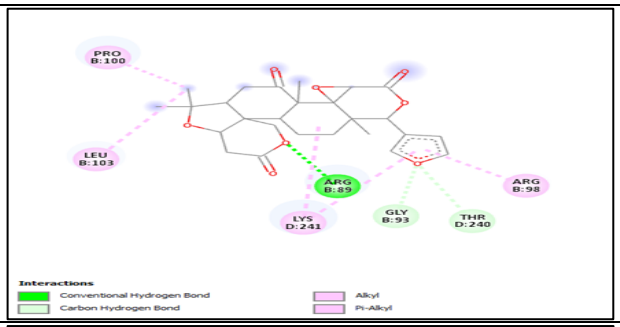
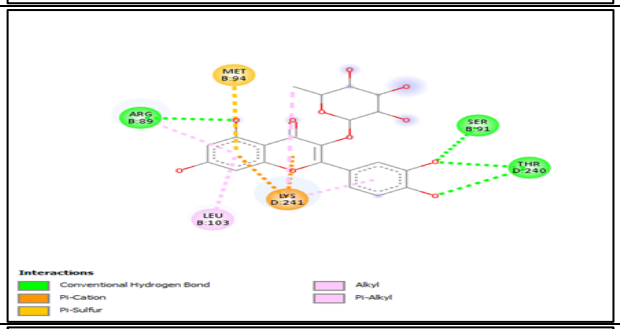
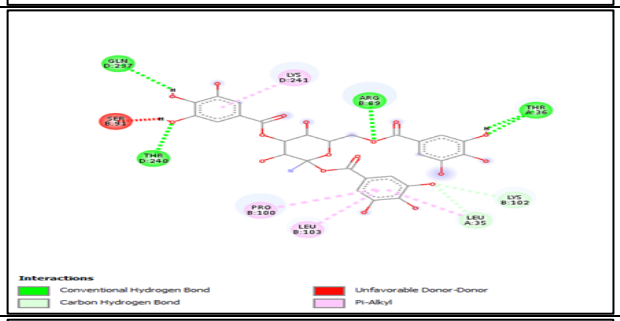
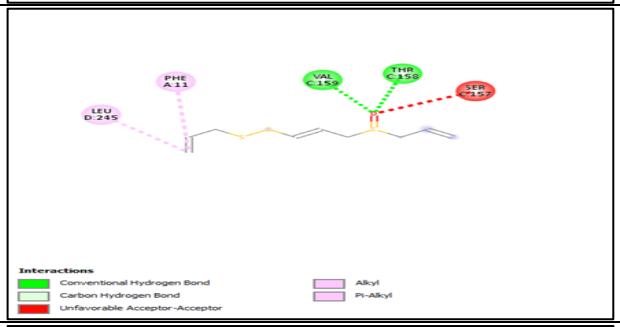
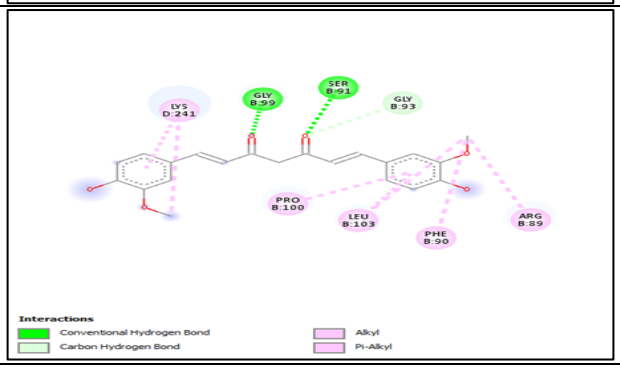
Limonin	-8.8	
Quercetin	-6.9	
Tannin amine	-7	
Ajoene	-4.7	
Curcumin	-7.8	

Table 5

Ligands	Acceptance	
	PDB ID-4OIG	PDB ID- 6VSO
Gingerol	Strongly accepted	Strongly accepted
Honokiol	Strongly accepted	Stronglyaccepted
Shogaol	Strongly accepted	Accepted
Thiosulfinate	Strongly accepted	Strongly accepted
Apigenin	Accepted	Strongly accepted
Baicalin	Accepted	Accepted
Coumarin	Accepted	Strongly accepted
Limonin	Accepted	Accepted
Quercetin	Accepted	Strongly accepted
Tannin amine	Accepted	Strongly accepted
Ajoene	Strongly accepted	Strongly accepted
Curcumin	Accepted	Accepted

Conclusion:

All the twelve ligands were studied using Bioavailability Radar. Our result proposed Gingerol, Honokiol, Shogaol, Thiosulfinate and Ajoene showed the best docking result for Dengue Virus Proteins with PDB Id 4OIG. While, Gingerol, Honokiol, Thiosulfinate, Apigenin, Coumarin, Quercetin, Tannin amine and Ajoene showed the best docking result for Dengue Virus proteins with PDB ID 6VSO.To find the effectiveness and to propose the exact mechanism In-Vitro Studies can be encouraged on Gingerol, Honokiol, Shogaol, Thiosulfinate, Ajoene, , Apigenin, Coumarin, Quercetin and Tannin amine targeting respective disease that are discussed above to understand the mechanism and potential cure for Dengue Virus Disease.

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