SUPPLEMENTARY MATERIAL

Antiviral phytochemicals identification from *Azadirachta indica* leaves against HCV NS3 protease: An In-silico approach

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Abstract: Hepatitis C virus is the major health problem across the world affecting the people of all age groups. It is the main cause of hepatitis and at chronic stage causes liver cirrhosis and hepatocellular-carcinoma. Various therapeutics are made against HCV but still there is a need to find out potential therapeutics to combat with virus. The goal of this study is to identify the phytochemicals of *Azadirachta indica* leaves having antiviral activity against HCV NS3 protease through molecular docking and simulation approach. Results show that the compound 3-Deacetyl-3-cinnamoyl-azadirachtin possesses the good binding properties with HCV NS3/4A protease. . It can be concluded from this study that Deacetyl-3-cinnamoyl-azadirachtin may serve as potential inhibitor against NS3/4A protease.

Keywords: HCV; NS3/4A protease; *Azadirachta indica*; molecular docking

1. Experimental

This study was designed to screen the phytochemicals of *Azadirachta indica* (neem) leaves against HCV NS3/4A protease. Intel [R] xenon [R] CPU-E5620 @ 2.40 GHz system having RAM-3.8 GB and have 11.4 (X 86_64) operating system was used for molecular docking. Protein ligand docking was performed with MOE (Molecular Operating Environment) software. Protein-ligand interactions were analyzed by ligPolt feature of MOE and Chimera was used to create interactions images.

1.1 Ligand Structure Preparation

Structures of neem phytochemicals were obtained from PubChem database (<https://pubchem.ncbi.nlm.nih.gov>), MAPS database (<http://mapsdatabase.com>), MP3D Database (<http://bioinform.info>) and few structures were drawn using ChemDraw software. All the 2D chemical structures were converted into 3D format by adding hydrogen atoms and energy minimizing was done with MOE having the parameters (MMFF94X force field, gradient 0.05). The minimized ligands were saved in .mol2 format. A database of all minimized structures was constructed and saved in .mdb format which was used as an input file for docking studies.

1.2 Protein Structure Preparation

HCV NS3/4A protease structure was retrieved from Protein Data Bank (PDB) (<http://www.rcsb.org/pdb/home>) with PDB ID-4A92. Water molecules were removed using MOE and hydrogen atoms were added to the receptor protein. 3D protonation and energy minimization was also carried out to optimize the receptor. Energy-minimization was done with the parameters (Gradient- 0.05, Force Field- MMFF94X+Solvation). Energy minimization was stopped when root mean square gradient reaches below 0.05. The minimized structure was used for docking.

1.3 Molecular Docking

Molecular docking was carried out using the ligand database against HCV NS3/4A protein by pocket selection from receptor and setting the docking-parameters through MOE. The output file was saved in .mdb. The top listed complexes having minimum S-score were checked to find the binding-interactions with active sites. The best orientations were considered for H-bond and π - π interactions by LigX in MOE.