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MOLECULAR DOCKING STUDY OF NATURAL COMPOUNDS AS NOVEL INHIBITORS OF NON STRUCTURAL PROTEINS (nsP2&nsP3) OF CHIKUNGUNYA VIRUS

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ABSTRACT
Chikungunya is a viral disease transmitted to human beings through mosquito bite. Chikungunya virus a member of the alphavirus genus belongs to the family *Togoviridae* and it is primarily transmitted to humans by two main vectors, *A. aegypti* and *A. albopictus.* Chikungunya can cause fever, chills, nausea, vomiting, joint pain, head ache, and swollen in the joints The CHIKV mortality rate has been estimated to be 1:1000 and most of the deaths occur in neonates, adults with underlying conditions and the elderly. The NonStructuralProtein2 have been described as virulence factors responsible for the transcriptional and translational shutoff in infected host cells and the inhibition of interferon mediated antiviral responses contributing to the controlling of translational machinery by viral factors. The NonStructuralProtein3 plays a major role during infection and in the assembly of stress granules. Hence an insilico attempt was made to characterize nonstructural proteins to identify the potential drug to inhibit the protein. The plant based natural compounds for chikungunya were used as natural ligands for molecular docking process with glide (version 5.6) of Maestro (10.2) (Schrodinger suite). In this study, Cis caffeic acid which is a Alkaloid isolated from caffeine belonging to Coffee (*Eucalyptus globules*) was found to have a good inhibitory effect on NonStructuralProtein2. Chlorogenic Acid was isolated from *Hibiscus sabdariffa* a green coffee beans plant have good inhibition effect on Non Structural Protein 3. These natural derived plant compounds were found to posses good binding affinity with Nonstructural proteins of chikungunya.

KEYWORDS: Chikungunya, Non structural proteins, molecular docking, Schrodinger suite.

INTRODUCTION
Chikungunya is a viral disease transmitted to human beings through mosquito bite. It enters the host cell by endocytosis. Chikungunya virus (CHIKV), a member of the alphavirus genus belongs to the family *Togoviridae* and it is primarily transmitted to humans by two main vectors, *A. aegypti* and *A. albopictus.* Chikungunya can cause fever, chills, nausea, vomiting, joint pain, head ache, and swollen in the joints. CHIKV was first isolated in the Makonde plateau in Tanzania during the earliest recorded epidemic in1953.CHIKV is believed to be maintained in a sylvatic transmission cycle between forest swelling mosquitoes and wild nonhuman primates such as vervet monkeys or baboons. A large outbreak of chikungunya has been reported in India between 2006 and 2007. Regrettably, *Aedes albopictus* is one of the world 100 most invasive species, increasing the risk of CHIKV emerging or re-emerging and becoming a major health problem around the world. (Saisawang *et al*., 2015).

The symptoms of CHIKF infection generally start 4–7 days after the mosquito bite. Infection presents in two phases, the first being acute, while the second stage is persistent (chronic), causing disabling polyarthritis. Acute infection lasts 1–10 days and is characterized by a painful high fever, asthenia, headache, vomiting, rash. Rash is the least reliable symptom, presenting in as few as 19%of patients. The mode of action of Chikungunya virus, by which it causes the disease remain to be investigated in detail and its mechanism of action has not yet been fully characterized accept the fact that it causes major histopathological changes in the skeletal muscle tissue, severe inflammation and necrosis of skeletal muscle.

The non structural protein2(3TRK) acts as protease and helicase, non-structural protein 3(3GPO) part of the replicase unit and an accessory protein involved in RNA synthesis, nsp4 RNA-dependent-RNA polymerase. The non-structural protein 2 of *alpha viruses* is a

multifunctional protein. The nonstructuralprotein2 is multifunctional whereby it possesses the N-terminal RNA triphosphatase domain which catalyses the capping of viral RNA as well as the proteins RNA helicase activity. The C-terminal of the protein has cysteine protease activity which is essential for the autocatalysis of the P123 polyprotein, as well as a domain with methyltransferase-like activity of undiscovered function. It has also been reported that nonstructuralprotein2 regulates the transcription and translation of viral proteins using the host cell machinery besides protection against interferons inhibitory activity.The function of *alphaviruses* nonstructuralprotein3 remains unknown, although mutations can affect different steps of the viral replication machinery. It is constructed of two domains, the first being a unique macro domain in the conserved N-terminal region. The C-terminal region is less conserved and is phosphorylated in approximately 16positions on serines and threonines. The function of phosphorylation is not understood, but it was found that deletion of these phosphorylated residues decreases the level of RNA synthesis. The N-terminus of nsp3 contains a macro domain which binds to ADP-ribose derivatives and RNA, and is able to hydrolyse ADP-ribose phosphate, a side product of cellular pre-tRNA splicing. Therefore, it is believed to control the metabolism of ADP-ribose phosphate and other ADP-ribose derivatives which have regulatory functions in the cell. The ADP ribose binding site within the nsp3 macro domain is solvent-exposed and points away from the other domains in the nsp23 polyprotein. Based on sequence conservation in *alphaviruses*, it has been that residues just after the nsp3 macro domain play a role in positioning of the nsp23 complex cleavage site. It can be inferred from the crystal structure of the nsp23 precursor protein of the closely related *alphaviruses*, that the nsp2 is connected to the nsp3 through the macro domain of the nsp3.

The nonstructuralprotein3cleavage site is located in a narrow cleft formed between that is inaccessible for proteolysis, and all the non-cytopathic mutants lie at the interface between nonstructuralprotein2 and nonstructuralprotein3. The inaccessibility of the nsp23 cleavage site indicates that access is tightly regulated. It is believed that the activator segment is located in the amino-terminus of the nsp2 which becomes exposed after cleavage from the nsp12 precursor poly protein. The moiety showed the strongest interactions with these residues in the enzyme pocket. Also, the ribose (with Thr111) and the diphosphate (with Val 33, Ser110, Gly112, Val113, Tyr114) units were found to play major roles in the CHIKV nsp3 ADP-ribose complex. This protein plays a major role during infection and in the assembly of stress granules. Stress granules are membranous cytoplasmic focal structures (foci) that immediately aggregate in response to cellular stress. This last action leads to impaired translation of most mRNAs. These stress granules may have antiviral activity that is inhibited by CHIKV replication by the nsp3 SH3 domain-binding motif.

The invasion of susceptible cells by the CHIKV is performed by two viral envelope proteins, E1andE2. Both carry the basic antigenic determinants and form the icosahedral shell of the virion particle**.** E1 and p62 peptide are type I membrane proteins and are derived from a structural polyprotein precursor. They are translated in the infected cell endoplasmic reticulum, into ap62–E1 heterodimer and processed by the Golgi. E3 protects the E2-E1 heterodimer from premature fusion with cellular membranes. The heterodimers trimerize forming the viral spikes. Cleavage of p62 into E3 and E2 during transport to the cell surface prepares the spikes for the fusogenic activation to enter the cell. At the plasma membrane, the formed virions bud through interactions between E2 and genome-containing viral nucleocapsids in the cytoplasm. In a recent study, the roles of four amino acid residues (G91, V178, A226, and H230) in the CHIKV E1 protein were linked to the E1 and cell fusion process. The study revealed that the highly conserved amino acid residues, G91 and H230, were important for membrane fusion functionality. The glycine residue (G91) is critical for the fusion process whereas any mutation or substitution in this residue lead to complete loss of E1 fusion ability. The E1 histidine is located outside of the fusion sequence, but still critical for the fusion. Other structural proteins also affect the E1 fusogenic capacity the E2 protein facilitates both E1 folding and regulatesE1 fusogenic properties in a pH and cholesterol dependent process. As an *alphavirus* family member, the hydrophobic fusion peptide of the CHIKV was found to be a trimer of hairpins composed of *β*-sheets in the post fusion state (type II fusion proteins) crystal structure of the CHIKV fusion peptide, consisting of 18 amino acid residues, which are residues in the full-length E1 glycoprotein. (Adel Rashad *et al*., 2014).

Molecular docking is the process by which two molecules fit together in 3Dimentional space to predict the binding modes of a ligand with a protein of known sequences. Docking problem is concerned with generation and evaluation of possible structures of protein-ligand complexes. The goal of ligand-protein docking is to predict the predominant binding model(s) of a ligand with a protein of known three dimensional structures. The main idea is to dock a database of potential metabolites into a proteins binding site and then rank them based on their calculated binding affinities. Due to approximation of the simulation, the calculated binding affinity, by itself, is used to identify a proteins cognate ligand amongst a crowd of candidates, and this approach can still be useful to experimentalists by narrowing down the number of molecules that need to be tested *invitro* condition. The objective of the study was based on screening of compounds have been performed using natural plant derived compounds. Docking analysis was carried out using Maestro (10.2) (Schrodinger suite) between these screened compounds and two protein

targets viz. Non-structural protein 2(nsp2), Non-structural protein 3(nsp3), Structural E1 protein (Envelope protein) Structural E2 protein (Envelope protein). Plant derived natural compounds were found to be best hits for protein targets. These natural compounds were found to possess good binding affinity with these proteins and considered to be effective drug targets for treatment of Chikungunya.

The present study was based on screening of compounds using natural plant derived compounds. Docking analysis was carried out using Maestro (10.2) (schrodinger suite) between these screened compounds viz. Non-structural protein 2(nsp2), Non-structural protein 3(nsp3). The screened compounds are Chikungunya inhibitors which are plant derived inhibitors found to possess good binding affinity with these effective drug targets for treatment of chikungunya.

MATERIALS AND METHODS

Proteins

The Protein Data Bank (PDB) (www.rcsb.org) is a worldwide repositoy for processing and distribution of 3D biological macromolecular structure data. The non structural protein 2 and non structural protein 3 were downloaded from Protein Data Bank.

Protein name PDB Entry

A. Non structural protein 2 Protease 3TRK
B. Non structural protein 3 3GPO

Ligands

Natural compounds

The natural compounds were collected from plant sources.. The plant derived sources such as flavonoids, terpenoids, Alkaloids, Miscellaneous plant derived natural compounds are considered as natural ligands. Fifty plant derived natural compounds were considered as ligands for molecular docking.

Molecular Docking

Molecular docking analysis was performed using Maestro, version 10.4 schrodinger suite. The following are the steps required for docking process.

PREPARATION OF PROTEINS

Using the protein preparation wizard of maestro provided by schrodinger the Non structural protein 2 and Non structural protein 3 was prepared and subjected for docking studies. Both the proteins are consists of water molecules, co-factor and Metal-ions. Docking process required bond orders assigned, non-structural protein preprocess was carried out. The waters and hetero atoms present in the structure were deleted. Optimization and minimization of protein was carried out using OPLS-2005-all-atoms force field.

Preparation of ligand

Totally fifty ligands was sketched using maestro and ligand preparation was done with Lig prep(version6.7), Implicit hydrogen atoms ware added to the compounds, and the geometrics were optimized by Macromodel. Epik program(version 2.1), was used for generation of ionization states of the compounds in the PH range 7± 2. After lig prep was finished, ionization states of the compounds were generated.

Evaluating for ADME

Quik prop

Quik prop is a quick, accurate, easy to use absorption, distribution, metabolism and excretion (ADME) prediction program present in a schrodinger suite. *In silico* ADME properties and structural descriptors were predicted using the program Quik prop(version-4.4).

Molecular docking

Schrodinger implements docking studies using Glide (version-6.7). Glide runs the docking in two steps:

- Receptor grid generation
- Ligand docking

Receptor grid generation

Binding site analysis was carried out with the sitemap program and the receptor grids were generated for corresponding binding sites of the proteins. The Non structural protein 2 and Non structural protein 3 is done form the receptor grid.

The grids are defined with the dimensions of 10Å in all the X,Y, and Z axis, selecting the centroid of the binding site as the centre of the grid box. Other parameters such as vander walls radii scaling factor and partial charge cutoff were set as default, 1.0 and 0.25 respectively for grid generation.

Ligand docking

Ligand docking was performed using XP mode (Extra precision) of Glide program. The docking results were viewed using glide XP visualize. Using XP visualize hydrogen, hydrophobic, electrostatic interactions can be visualized.

RESULTS

Proteins

The crystal structures of the structural envelope protein of nsP2 protease and nsP3 were downloaded from protein data bank.

Ligands

Natural compounds collected from various plant derived sources such as flavanoids, Alkaloids, clove, grass. Natural compounds were collected from various medicinal plants which possess anti viral activity. These compounds were sketched using Maestro.

Molecular docking

Molecular docking analysis was performed using Maestro, version 10.2, Schrodinger suite. The steps involved in docking Process are as follows:

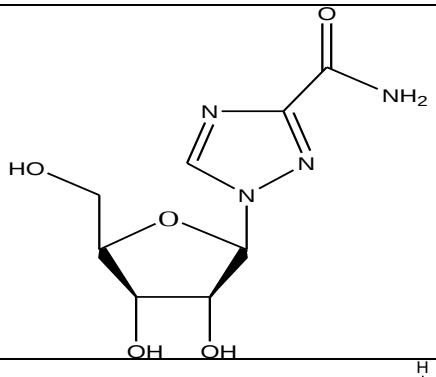
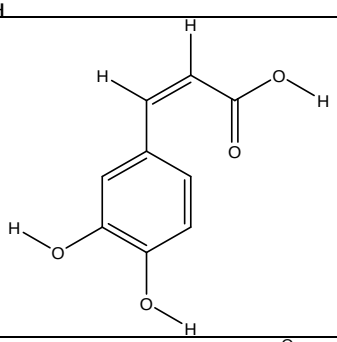
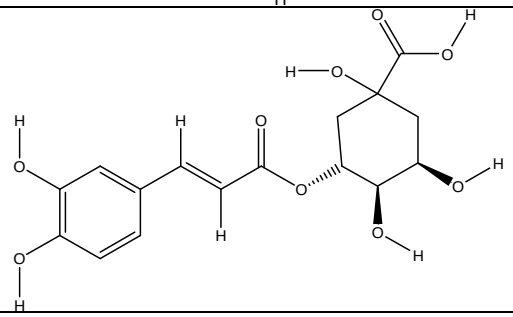
Preparation of proteins

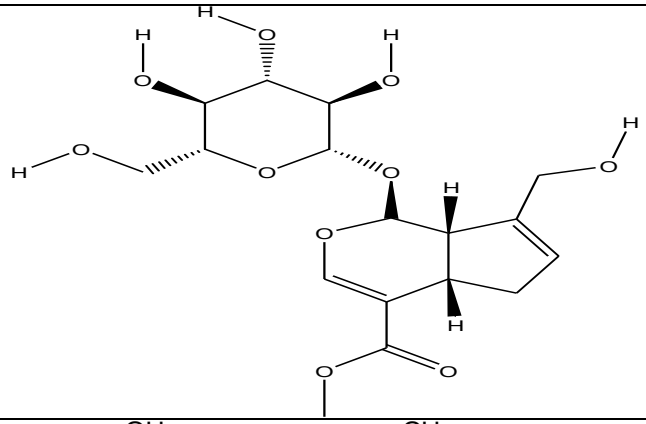
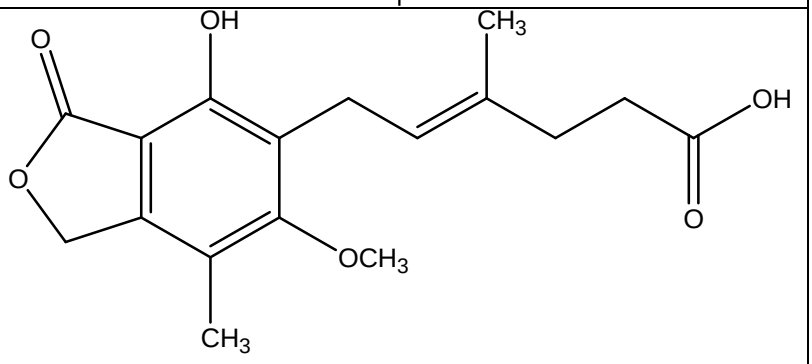
To obtain the assigned NonStructuralProtein2 (3TRK), NonStructuralProtein3 (3GPO), the protein preparation was carried out using the protein preparation Wizard of Maestro Version 10.2 provided by Schrodinger. NonStructuralProtein2 (3TRK) consists of A chain and these proteins posses water residues. Nonstructural protein 3(3GPO) Consists of A, B Chain and these proteins posses water residues. All water residues were deleted because no water residues were bound with the protein. The sodium metal ions were not deleted. Proper bond order of the protein was assigned and all hetero atoms and other unwanted chemical moieties were deleted during protein preparation. Optimization and minimization of protein was carried out using OPLS-2005 all-atoms force field. Optimization and minimization of protein was carried out using OPLS-2005 all-atoms force field. Optimization is carried out to optimize the protein hydrogen bond which greatly decrease the protein preprocess preparation of time. Minimization was done to allow hydrogen bonds to be freely minimized and it helps sufficient, heavy atoms movement to relax strained bonds and angle.

Preparation of ligand

The ligands such as plant natural compounds for non structural protein 2 protease and non structural protein 3 After ligands preparation implicit hydrogen atoms were added to the ligands and theGeometries were optimized. Number of ionization states was generated for every ligand by Epikprogram (version 5.6).For fifty compounds derived from plant sources. The best hits of natural compounds derived from plant sources are indicated in below table

Table 1: Chemical structures of lead molecules.

Ribavirin	
Cis caffeic acid	
Chlorogenic acid	

Geniposide	
Mycophenolic acid	

Qik prop

The absorption, distribution, metabolism and excretion ADME properties of all natural compounds for non structural protein 2 Protease (3TRK) and non structural protein 3 (3GPO) were predicted using Qikprop (version 4.4). All naturally available ligands were predicted to have very good ADME properties and no compounds were failed and skipped during the qikprop.

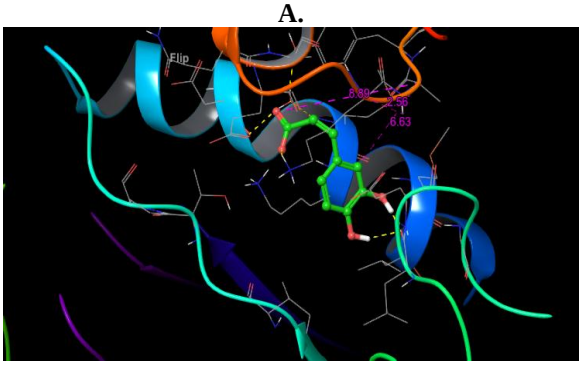
Receptor grid generation

Docking studies were performed using Glide, version(6.7). Non Structural envelope protein 2protease (3TRK) and non structural protein3(3GPO) of has natural ligands so protein site mapping (version3.5) is not done to form the receptor grid. This protein has natural ligands and hence site mapping is not done to form the receptor grid. This receptor grid act as the active site for these respective proteins.

Ligand docking

The Ligand docking was Performed using XP mode(Extra precision) of Glide program. The docking process was made between natural compounds with non structural Envelope protein 2 protease-1(3TRK).Cis caffeic acid is a Alkaloide isolated from caffeine belonging to Coffee (*Eucalyptus globules*) plant natural derivatives in has become the first hit with a glide score of -6.73.Cis caffeic acid against to Non structural protein 2 Protease(3TRK) protein.

A.



B.

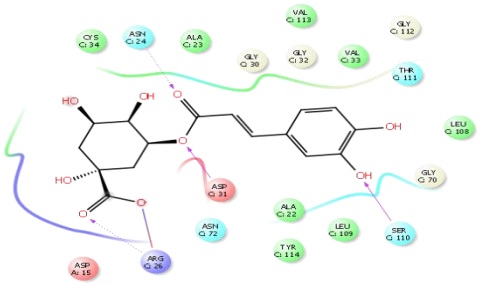
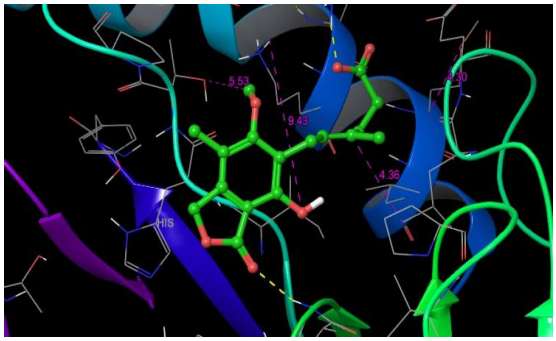


Figure 1: (A) Ligand Interactions diagram between Active site residues of non Structural Envelope protein 2 protease and cis caffeic acid. (B). Ligand interaction diagram of lead compound cis caffeic acid with nsP2 protease (PDB ID: 3TRK).

Mycophenolic acid is a Antibacterial compound abundant in spoiled corn and isolated from the fungus(*Penicilium glaucum*) A plant natural derivative

has become the Second hit with a glide score of -5.64. Mycophenolic acid against to Non structural protein 2 Protease(3TRK) protein**.**

A.



B.

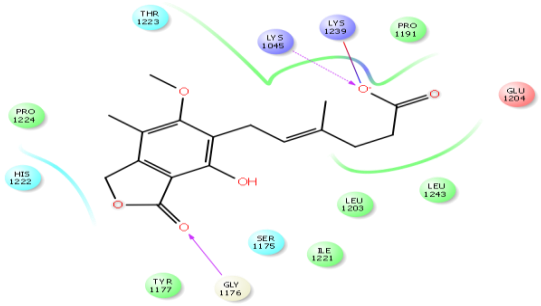
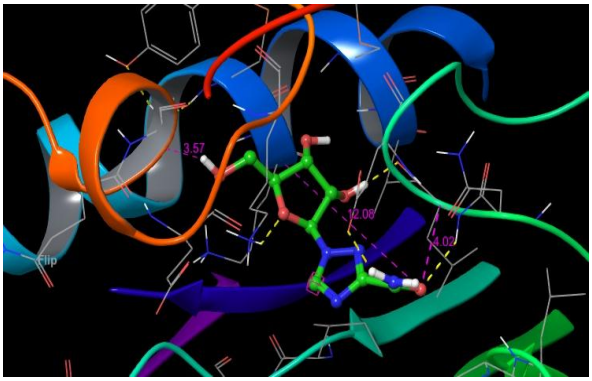


Figure 2: Non structural protein 2 protease (3TRK) protein and Mycophenolic acid.(A)Ligand Interactions diagram between Active site residues. (B) Ligand interaction diagram of lead compound Mycophenolic acid With nsP2 Protease (PDB ID: 3TRK).

Ribavirin is Antibiotic compound derived from the (3-carboxamidine). Ribavirin A *Plant* natural derivative has become the third hit with a glide score of -5.41. Ribavirin against to Non structural protein 2 Protease(3TRK) protein.

A.



B.

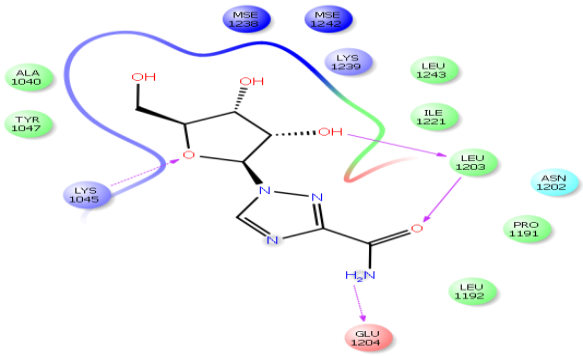
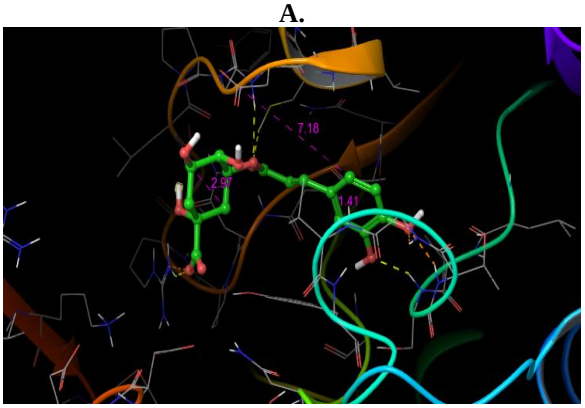


Figure: 3 Non structural protein 2 protease (3TRK) protein and Ribavirin.(A)Ligand Interactions diagram between Active site residues. (B) Ligand interaction diagram of lead compound Ribavirin With nsP2 Protease (PDB ID: 3TRK).

Then the docking process was made between natural compounds with Non structural protein 3 - 2(3GPO) protein. Chlorogenic acid *is a Alkaloid isolated from caffeine belonging to Coffee (Eucalyptus globules) plant natural derivatives* in has become the first hit with a glide score of -10.21.Chlorogenic acid against to Non structural protein 3(3GPO) protein.

A.



B.

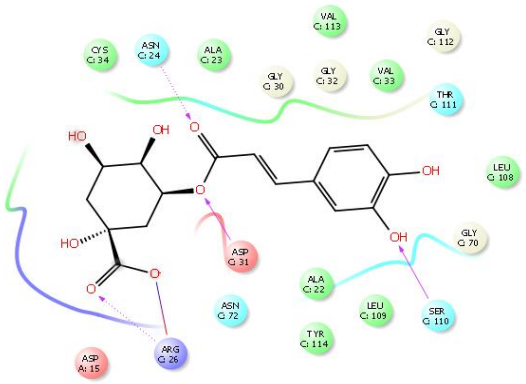
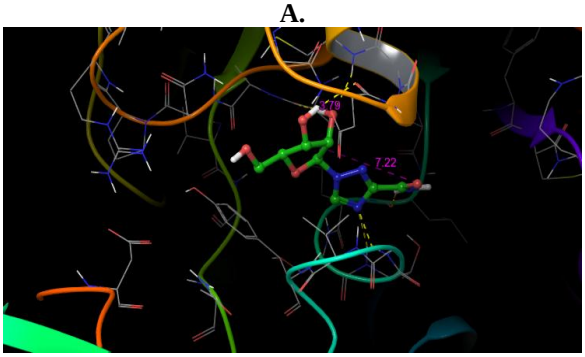


Figure 4:(A) Ligand Interactions diagram between Active site residues of non structural protein 3 and chlorogenic acid.(B)Ligand interaction diagram of

lead compound chlorogenic acid with nsP3 (PDB ID: 3GPO).

Ribavirin is derived from Antibacterial compound from the (3-carboxamidine). A *Plant* natural derivative has become the second hit with a glide score of -10.12. Ribavirin against to Non structural protein 3(3GPO) protein.

A.



B.

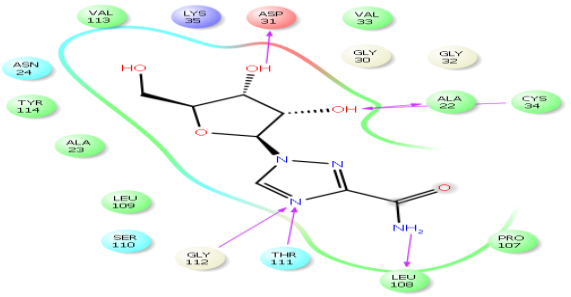
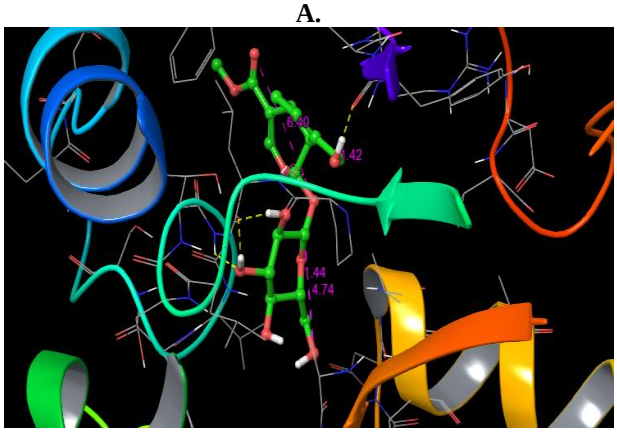


Figure 5: (A)Ligand Interactions diagram between Active site residues of non structural protein 3 and Ribavirin.(B)Ligand interaction diagram of lead compound Ribavirin with nsP3 (PDB ID: 3GPO).

Geniposide is a Alkaloide isolated from Gardenia fruit (*Gardenia jasminoidenoides*). This is plant based natural *derivative and* has become the third hit with a glide score of -9.54 against Non structural protein 3(3GPO) protein.

A.



B.

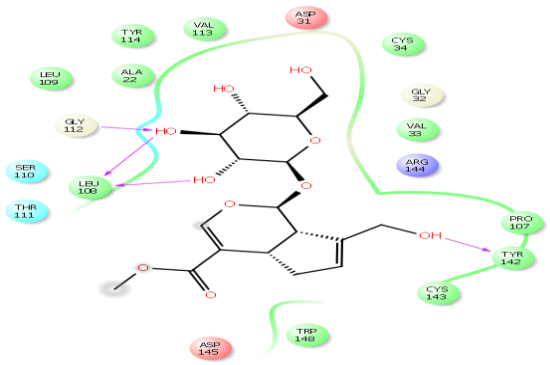


Figure 6(A): Ligand Interactions diagram between Active site residues of non structural protein 3 and Geniposide.(B)Ligand interaction diagram of lead compound Geniposide with nsP3 (PDB ID: 3GPO).

The results of docking exhibits natural compounds have best inhibitory effect on non structural protein 2 protease (3TRK) with their glide scores. Among Fifty compounds screened the first hit was Cis caffeic acid which is a Alkaloide isolated from caffeine belonging to Coffee (*Eucalyptus globules)* a plant natural derivatives has proved to be best hit for inhibiting the antigenic activity of non structural protein 2 protease(3TRK). Best three hits were having the inhibitory antigenic activity such as Cis caffeic acid, Mycophenolic acid and Ribavirin. The other natural compounds such as Dephnadorin, Alpha mangostin, Gamma mangostin, Eriodictyol, Chlorogenic acid, Phyllamyricin. B, Daucostrol, Cyanopurin, Hispidulin, Arbitol,Apigenin, Kaemferol,Piperine, Chrysin, Isocolumbin, Cacalone, Cacalol,Costunolide, Germacrene, Chemiquinone, Lycopodine, Supinine, Goniotholamine, Dehydrocostunolide. have good binding affinity to both Non structural protein 2 Protease and Non structural protein 3 proteins. Screening process of these compounds was done by using Maestro (Version 10.2), a Schrodinger suite. Plant based Natural compounds binds to the active sites of Non structural protein 2 Protease and Non structural protein 3 Proteins and exhibits, higher binding affinity with these drug targets.

Table 2: Glide score for natural compounds docked with (non structural protein 3) protein.

S.NO	LIGAND NAME	GSCORE
1.	Chlorogenic acid	-10.21
2.	Ribavirin	-10.12
3.	Geniposide	-9.54
4.	Dyphylin	-9.26
5.	Chrysin	-8.58
6.	Kaemferol	-8.55
7.	3-o methyl quercitin	-8.45
8.	Hispidulin	-8.28
9.	Alpha mangostin	-8.1
10.	supinine	-7.94

Table 2: Glide score for natural compounds docked (non structural protein 2 Protease) protein.

1.	Cis caffeic acid	-6.73
2.	Mycophenolic acid	-5.64
3.	Ribavirin	-5.41
4.	Dephnadorin.A	-5.27
5.	(2R,3S,4S,5R)-5-(6-aminopurin-9-yl)-3,4-dihyroxyoxolan-2-yl]methyl sulfate, tetrabutyl azanium	-5.26
6.	Geniposide	-5.2
7.	Alpha mangostin	-4.89
8.	Eriodictyol	-4.8
9.	Chlorogenic acid	-4.69
10.	Phyllamycin. B	-4.14

CONCLUSION

Non structural protein 2 Protease (nsp2) and Non structural protein 3(nsp3) are involved in the chikungunya infection and can be considered as the best drug targets of chikungunya disease. A docking model for these proteins have been developed with Glide 6.7, an automated docking program, successfully reproduced the binding mode of crystal structures of Non structural protein 2 Protease and Non structural protein 3 inhibitors. Various medicinal plant derived natural compounds have been screened in order to find out the best leads. From this study it was concluded that, was Cis caffeic acid , Mycophenolic acid and Ribavirin was proved to be the naturally available novel inhibitors for Non structural protein 2 Protease. For Non structural protein 3Chlorogenic acid, Ribavirin and Geniposide compoundshas High Binding Affinity with drug targets.

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