CHAPTER 4

RESULTS

4.1 Multiple Sequence Alignment of Flaviviruses

Clustal Omega online tool was used to align the flaviviruses sequences. Sequences used as shown in Table 3.1. In this study, 5 sequences were used. Out of the 5 sequences, 3 were obtained from PDB database while 2 were obtained from NCBI database.

The sequences were aligned and it was found that the catalytic triads are found at the conserved regions which are located at the NS3 protease. The catalytic triad residues: His51, Asp75 and Ser135, are highlighted in yellow colour and are found in boxes (Figure 4.1). The regions in boxes are identified as significant similarity surrounding the catalytic triad residues and residues that might form the substrate-binding pocket. Figure 4.1 shows the multiple sequence alignment of Dengue Virus (DEV), West Niles Virus (WNV), Japanese Encephalitis Virus (JEV), Yellow Fever Virus (YFV) and Hepatitis C Virus (HCV) sequences in which the level of residue conservation is relatively high at the putative catalytic residues or homology boxes.

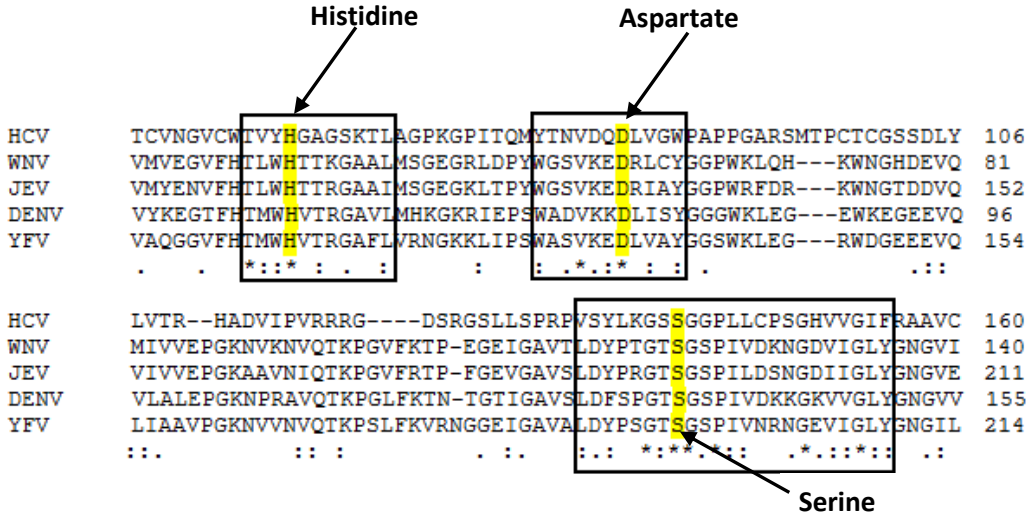

Figure 4.1: Multiple Sequence Alignment of Flaviviruses.

4.2 Identification of anti-viral target binding site through literature research

Literature research of several journals also support the findings of multiple sequence alignment which shows that binding sites of serine proteases consists of Histidine, Aspartate and Serine residues. The residues that consists catalytic triad of HCV pro (His57, Asp 81 and Ser139) have equivalents in His51, Asp75 and Ser135. The triads come along a cleft formed at the interface between the two domains although these stated residues are located in different protein domains (Rose et al., 1999). Other flaviviruses also reported to have the conserved regions at these triads. PyMOL viewer enables the user to view the protein structures with preferred display to show clearly the possible binding sites. The structure of the flaviviruses with different displays is reported in this study for the understanding of binding sites of the viruses. Figure 4.2, 4.3, and 4.4 shows the representation of West Niles Virus (PDB ID: 2FP7). The binding sites are coloured in red and labeled.

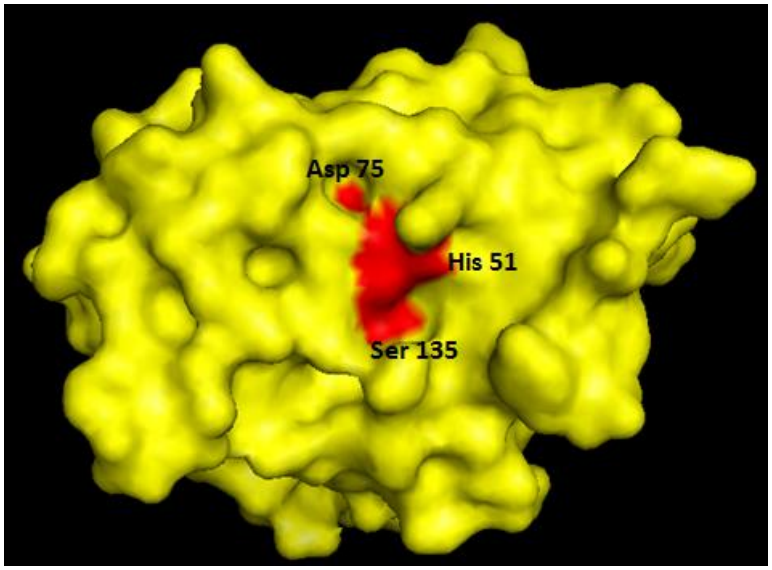

Figure 4.2: Surface Representation of classic His-Asp-Ser catalytic triads (His 51, Asp 75 and Ser 135) in West Niles Virus

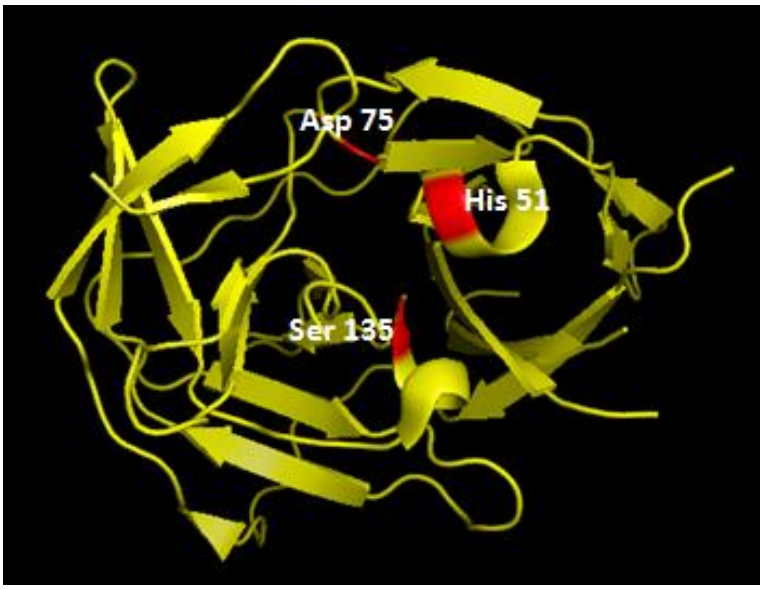

Figure 4.3: Cartoon Representation of classic His-Asp-Ser catalytic triads (His 51, Asp 75 and Ser 135) in West Niles Virus

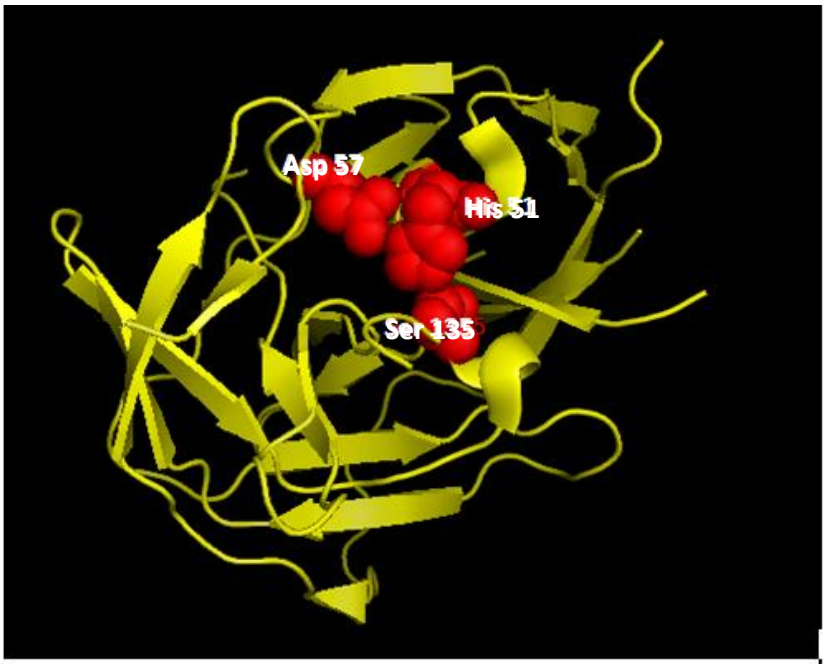

Figure 4.4: Cartoon Representation of West Niles Virus structure with the catalytic triad represented in spheres.

Precise arrangement of this classic triad is required to enhance the nucleophilicity of the serine hydroxyl group.

The anti-viral binding sites of Hepatitis C (PDB ID: 4I31) are Histidine 57, Aspartate 81, and Serine 139. The binding sites are coloured with red and showed in different displays in figure 4.5, 4.6 and 4.7.

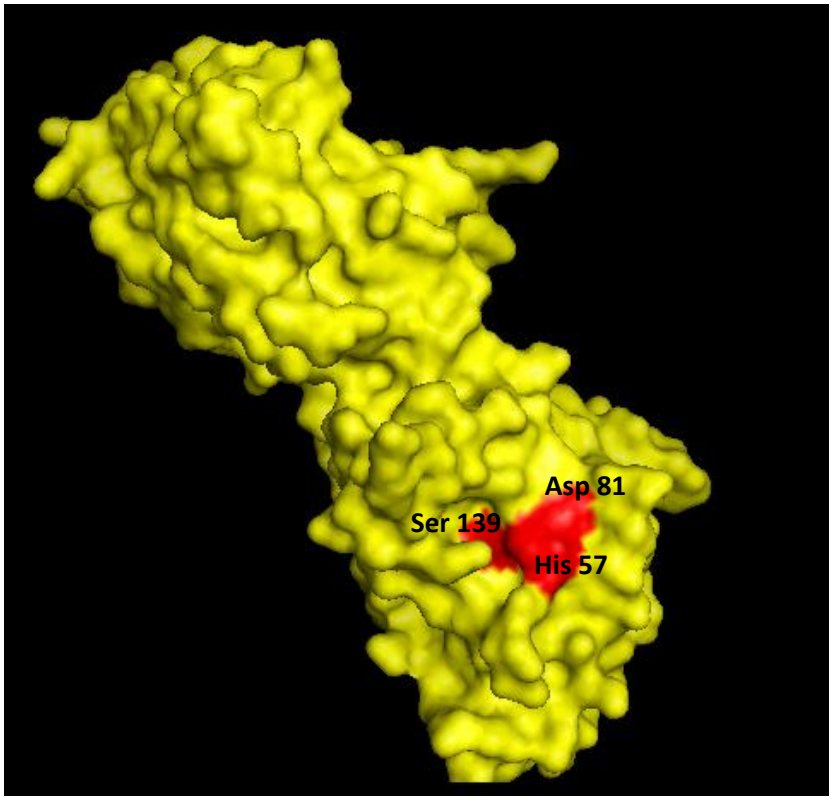

Figure 4.5: Surface Representation of a His-Asp-Ser catalytic triads(His 57, Asp 81 and Ser 139) in Hepatitis C Virus

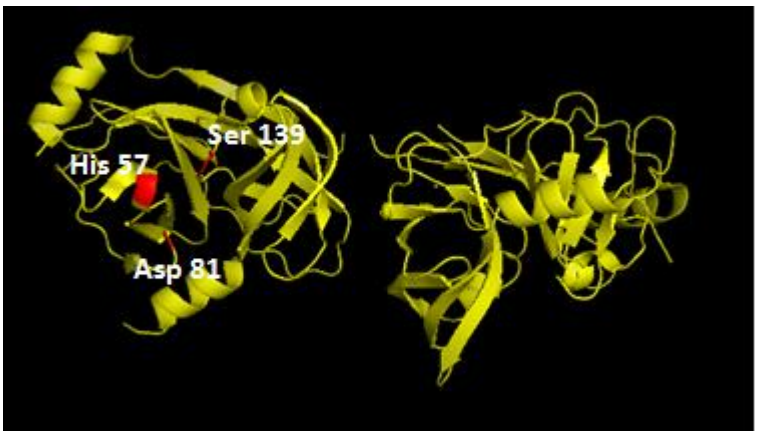

Figure 4.6: Cartoon Representation of a His-Asp-Ser catalytic triads(His 57, Asp 81 and Ser 139) in Hepatitis C Virus

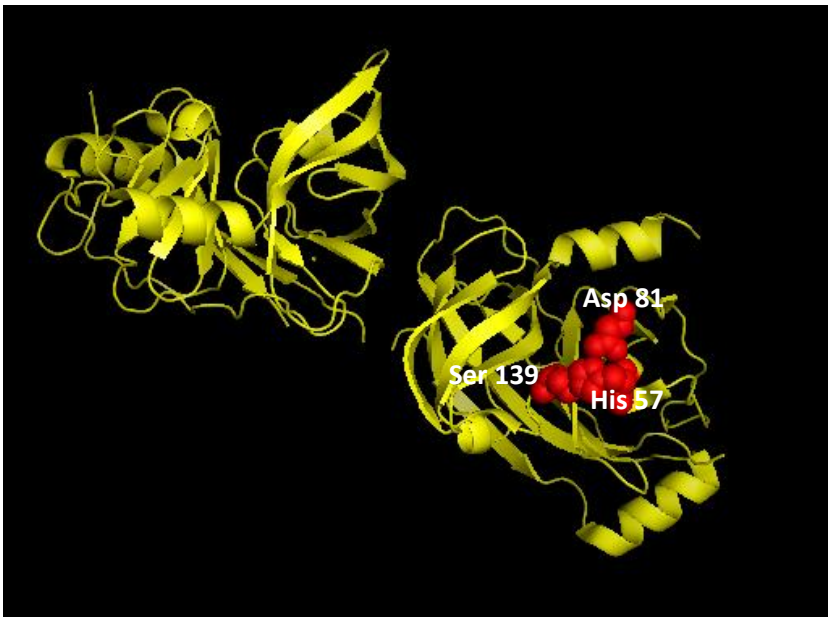

Figure 4.7: Cartoon Representation of Hepatitis C Virus structure with the catalytic triad represented in spheres.

The structure of Dengue Virus (DENV) reveals a substrate binding cleft that is small and shallow made of catalytic triads (Histidine 51, Aspartate 75 and Serine 135). Figure 4.8, 4.9 and 4.10 shows the representation of Dengue Virus (PDB ID: 2FOM). The binding sites are coloured in red and labeled.

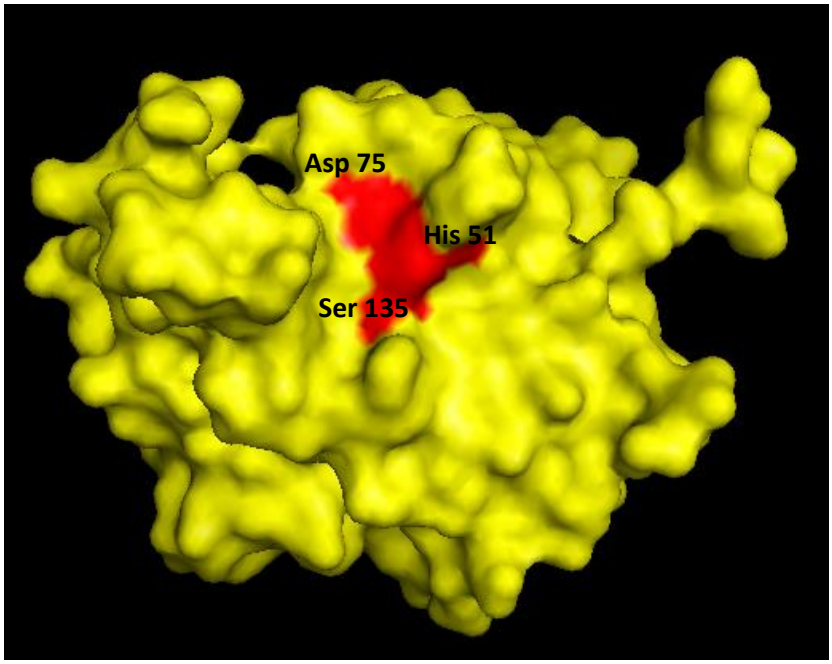

Figure 4.8: Surface Representation of a His-Asp-Ser catalytic triads (His 51, Asp 75 and Ser 135) in Dengue Virus

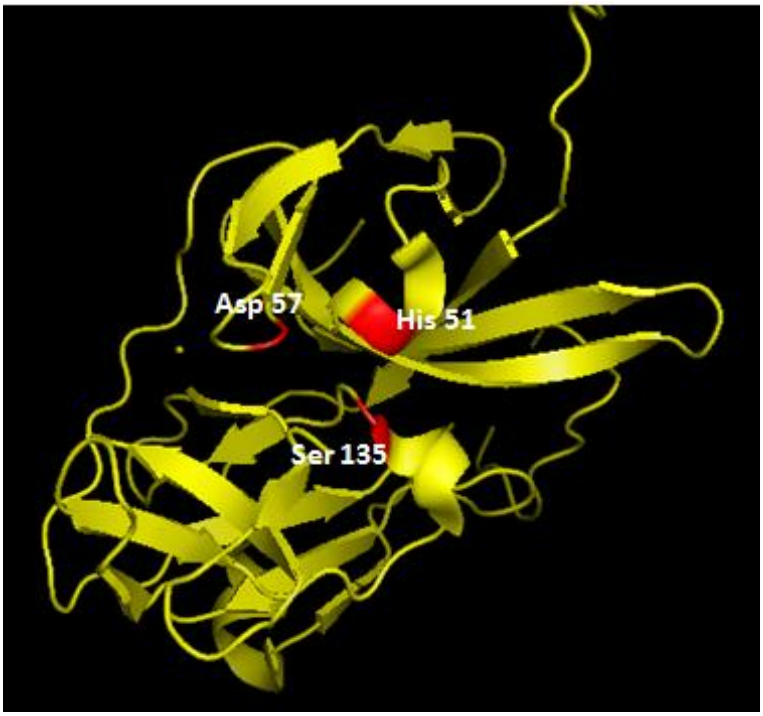

Figure 4.9: Cartoon Representation of Dengue Virus structure with the catalytic triad (His 51-Asp 75- Ser 135).

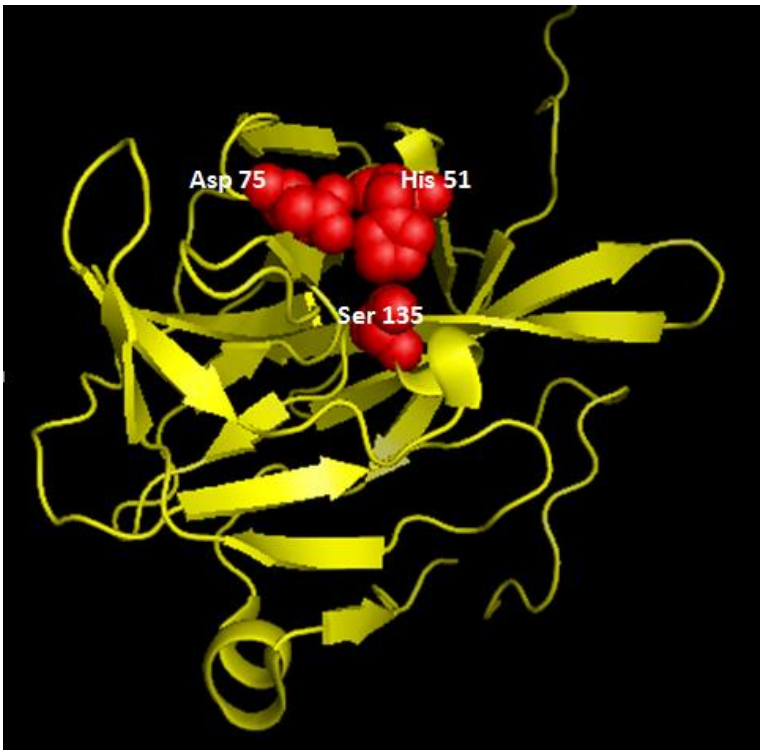

Figure 4.10: Cartoon Representation of Dengue Virus structure with the catalytic triad represented in spheres.

The protein structure of Yellow Fever Virus (NCBI Accession Number: NP_776005) and Japanese Encephalitis virus (NCBI Accession Number: NP_775670) is still not available in any protein structure databases and the identification of binding sites are based on the multiple sequence alignment performed on the retrieved sequences.

Table 4.1: The identified binding sites of Flaviviruses

SPECIES	**PDB ID/ NCBI Accession Number**	**IDENTIFIED BINDING SITES**
YELLOW FEVER VIRUS (YFV)	NP_776005	His 109, Asp 133, Ser 194 (triads)
JAPANESE ENCEPHALITIS VIRUS (JEV)	NP_776570	His 107, Asp 131, Ser 191 (triads)
WEST NILE VIRUS (WNV)	2FP7	His 51, Asp 75, Ser 135 (triads)
HEPATITIS C VIRUS (HCV)	4I31	His 57, Asp 81, Ser 139 (triads)
DENGUE VIRUS (DENV)	2FOM	His 51, Asp 75, Ser 135 (triads)