# CHAPTER 5

# DISCUSSION

## 5.1 MULTIPLE SEQUENCE ALIGNMENT OF FLAVIVIRUSES

Bollati et al., 2010 states that enzymatic activities necessary for RNA capping and genome replication is performed by NS3 proteins in flaviviruses. Multiple sequence alignment using Clustal Omega online tool is performed to analyze sequence conservation of the involved flaviviruses sequences. The alignment of the flaviviruses sequences shows that residues which are important for encoding of viral serine protease are conserved in NS3. The catalytic triad of histidine, aspartate and serine residues required for serine protease activity ( Polgar, 2005 ) was conserved in the flaviviruses sequences as shown in **Table 4.1**. These findings can lead to the secondary structure prediction of anti- viral binding sites and a strong correlation can be made between regions with highly conserved sequences across the different proteases and structurally conserved regions. The recognized triads are important in unwinding of the RNA strand (Dumont et al. 2006 ) and to facilitate the initiation of viral replication. The active sites revealed in this study are revealed based on previous studies and also multiple sequence alignment which is homology to each other. Multiple sequence alignment clearly states that the amino terminal domain contains the serine protease catalytic triads consisting amino acid residues His51, Asp 75 and Ser 135. It is notable that the binding sites of Hepatitis C which are His 57, Asp 81 and Ser139 equivalents with His51, Asp75 and Ser135.

## 5.2 IDENTIFICATION OF BINDING SITES THROUGH LITERATURE RESEARCH

Bazan & Fletterick (1989, 1990) reported that structural motifs as well as the catalytic triad (His-Asp-Ser) of mammalian serine proteases were conserved in all flaviviruses.

The structures for Yellow Fever Virus (Accession Number: NP_776005) and Japanese Encephalitis Virus (Accession Number: NP_775670) are still not available in the protein databases. However the sequences obtained from this accession number aligned well at the triad regions of the other flaviviruses sequences. Therefore, these sequences are considered as the correct sequences for this study. Rice, et al. (1985) also reported that the genome organization of this Yellow Fever Virus sequence (Accession Number: NP_776005) in his studies. This genome organization implies that posttranslational cleavage of a polyprotein precursor produces mature viral proteins. These have implications for flaviviruses RNA replication and for the evolutionary relation of this virus family to other RNA viruses. The overall amino acid sequence homologies for the FASTA sequence of Japanese Encephalitis Virus (JEV) with accession number: NP_775670 were 76.2% for JEV and WNV, 46.1% for JEV and YFV, and 46.0% for WNV and YFV (Sumiyoshi, et al., 1987).These results indicate a very close agreement with serological relations already proven among these viruses. The well-conserved regions in these viruses are similar and may perform important functions for viral proliferation. All these reasons lead to the choice of this JEV sequence being used in this study.

## 5.3 NON-STRUCTURAL PROTEIN NS2B- NS3 SERINE PROTEASE

Protease activity of NS3 is essential for viral replication. Thus, it's inhibition shall be considered as a principal intervention strategy for the treatment of flaviviral infections. The residues around triads in this domain are well conserved and shall be highly considered for the future studies of antiviral treatment of flaviviruses infection. The N terminal protease domain (NS3pro) is responsible for proteolytic processing of the viral polyprotein and the C terminal region with RNA triphosphatase RNA helicase and RNA- stimulated NTPase domain are important for RNA replication(Singh,J. et, al., 2011). Thus, NS3 protein is the preferred choice of study for the inhibition of viral proteases that stop the proteolytic processing and subsequently prevent viral infections in clinical settings. This proteolytic domain was made of roughly 180 amino acid residues with a catalytic triad conserved in all sequences, of His- Asp- Ser (Bazan & Fletterick, 1989, 1990; Gorbalenya et al., 1989). Like the protease domain, the helicase domain also show similar pattern of relatedness as was found for a region of the helicase domain of NS3 (Ohba et al., 1996). The found triads are responsible for the cleavage at the NS2A/2B and NS2B/NS3 sites in an apparent intramolecular fashion. (Pugachev et al., 1993).

## 5.4 LIMITATION OF THE STUDY

Throughout the course of this study, few limitations were encountered. There are protein structures of flaviviruses sequences which are not reported yet. The 3D structure for Yellow Fever Virus (YFV) and Japanese Encephalitis Virus (JEV) is not reported yet in the protein databases.