Virtual screening of phytochemicals that bind to dengue viral serotypes using molecular docking methods

Vijayakumar. S[a] , Ramesh. V[a], Prabhu. S[a], Nirajmohan. S[b], Manogar. P[a], Natanamurugaraj. G[c] and Vathsala Mohan[d*]

Abstract— Dengue is the most common mosquito-borne viral illness in humans. Globally 2.5-3 billion individuals living in approximately 112 countries experience dengue transmission and/or infection. Annually 50-100 million individuals are infected with dengue virus and, currently, there is no specific antiviral treatment available. This study aimed to identify novel inhibitors for dengue serotypes from plant-derived bioactive compounds using protein-ligand docking methods. Molecular docking analysis was targeted at the dengue virus E and NS proteins. Of the 12 ligands tested, the flavonoid chalcone had the highest binding score and had promising interactions with E proteins of all four dengue serotypes and NS1. Andrographolide, quercetin and pinostrobin were found to have similar interactions with DENV 1 serotypes while chalcone and quercetin (docking scores, -7.580 and -70337 respectively) were effective against DENV 2. Chalcone was the only compound that had the ability to be docked with DENV 3 E protein and had a docking score of –7.052 with DENV 4. In conclusion, our molecular docking experiments identified chalcone as a potential drug candidate for further investigation towards the development of anti-dengue viral drug and/or towards anti-dengue therapeutic purposes.

Index Terms—Bioflavonoids, Chalcone, Dengue virus, DENV, Molecular docking, Schrodinger suite, serotypes.

1 INTRODUCTION

Dengue infection has become a global health problem. This dreadful disease has affected almost 2.5 billion people [1] with an estimated number of 25,000 deaths per year [2]. Recent studies show that more than 100 countries and about 50-100 million people have been affected with this dreadful disease. Asia, Central and South America and Africa are the countries that are majorly affected by this virus [3]. Dengue fever is caused by a Flavivirus, a member of the family Flaviviridae containing four serotypes including DENV-1, DENV-2, DENV-3 and DENV-4 (Weaver et al., 2009). This virus is vector-borne transmitted by two species of mosquitoes, Aedes *aegypti* and *Aedes albopictus* to humans [4].

Dengue viral genomes possess a single-stranded RNA which is translated into a single polyprotein where the proteome contains three structural proteins and seven nonstructural proteins [5]. The structural proteins are capsid protein, C; membrane protein, M; envelope protein, E and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). The E proteins play a vital role in allowing the virus to enter into the host cell. Therefore, E proteins are the crucial major targets for drug development. Non-structural proteins are mainly involved in viral replication (Fig 1) (See Lim, Rahman [6]) that also plays an important role in infection as well as in drug development.

Dengue virus infection suppresses the immune system producing clinical signs including headache, inflammation, bleeding, hypertension and mental disorders where the infection culminates in death in some cases due to liver damage [7]. At present, there is no vaccine or effective drug available for the treatment of DENV infection [8]. Medicinal plants that contain naturally occurring phytochemicals [9] form an attractive alternative for developing drugs for dengue viral infection. These phytochemicals form the active components of the plants' immune system and these compounds have been shown to possess antimicrobial properties against human as well as animal pathogens and/or infections [10]. Medicinal plants contain a wide variety of phytochemicals such as organosulfur compounds, limonoids, furyl compounds, alkaloids, polyines, coumarins, thiophenes, peptides, flavonoids, terpenoids, polyphenolics and saponins that have demonstrated therapeutic functions against a wide range of viruses through scavenging, hampering viral entry and interrupting or terminating DNA/RNA replication [11]. Previous studies have reported that secondary metabolites derived from plants such as flavonoids, Chalcone derivatives, and bioflavonoids have shown inhibitory activities against the DENV2, NS2B-NS3 and serine proteases [12, 13]. Selected medicinal plants are the most reliable sources to provide useful leads in the usage of phytochemicals against human diseases and pathogens which can be sourced from traditional Indian medicine, Ayurveda, a specific branch of Indian Ethnomedicine [14]. Phytochemicals and medicinal plants have been known to

[a]Computational Phytochemistry Lab, P.G. and Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur district, Tamil Nadu, India-613 503.
[b]Faculty of Health and Medical Sciences, University of Western Australia, Perth, WA 6009, Australia
[c] Faculty of Industrial Sciences and Technology, University Malaysia Pahang, Malaysia.
[d] The New Zealand Institute of Plant and Food Research Limited, Mt.Albert Research Centre, Auckland, * Corresponding author Email: *lingvathsala@gmail.com*

play a pivotal role in treating human diseases for several decades in India and therefore this study has utilised the wealth of knowledge in phytochemicals and their potential role as antimicrobials for viral infection with special reference to Dengue [15]. With regards to phytochemical drugs, majorities have been used in treating human illnesses [16] and several others have been used in clinical drug development [17] and often these drugs can be traced back to their natural origins. India has a rich herbal repertoire which is used in traditional Ethnomedicine and these medicinal plants are preferred over non-Ayurvedic treatment due to their multiple target activities, negligible side-effects and cost effectiveness [1].

Recent computational advances have opened a new platform for novel drug developmental studies. The prediction of the predominant binding mode of a ligand with a protein of known three-dimensional structure (Molecular docking) is considered an important technique in drug designing and screening of novel antiviral compounds against challenging diseases [18]. Therefore, the present study was aimed to design and screen 12 bioactive compounds against dengue serotypes using *in silico* techniques. The main objective of this study was to target the hydrophobic pockets of E and NS proteins of the dengue serotypes with novel bioactive compounds that could help in the inhibition of dengue infection. The results from this study have shed useful insights into drug development and computer-aided screening of the drugs against DENV infection and the potential drug candidates against dengue infection using which further investigations can be carried out in the future.

2. MATERIALS AND METHODS

2.1. Computational methods with glide version 10.2

Computational studies were carried out using Schrodinger suite Maestro version 9.5, installed on a single machine running on Intel Core i7 Duo processor with 1GB RAM and 275 GB hard disk with Black Dell Inspiron version 7.0 as the operating system.

2.2. Sources of databases:

Although previous studies have reported that bioactive compounds have been used against DENV serotypes, in our present study, we chose a selected set of phytocompounds that can target the E and NS proteins of dengue for molecular docking. All protein (E and NS) sequences were retrieved from the NCBI gene bank database (www.ncbi.nlm.nih) (Accession no.: ACN42675.1, AHG23152.1, AEZ01357.1, AAG30148.1, AHG23165.1, AHF45700.1 and AAG15000.1). The bioactive ligands were collected through electronic databases appropriately as suitable (www.chemspider.com). Both molecules were prepared and validated using the energetic Schrodinger suite, Maestro 9.5 version.

2.3. Protein preparation

Selected targets were prepared using Protein preparation wizard tools from Schrodinger suite. This preparation involved removing water molecules, HET (heteromers) numbers and this tool was also used to find the missing side chains with updated missing amino acid residues on the targets. Preparation and refinement were done by running a ProteinPrep job on the structure using a standard procedure. Optimisation and Minimization of the proteins were performed until the average root mean square deviation of non-hydrogen atoms reached 0.3 Å.

2.4. Validation of binding site (SiteMap)

The SiteMaps were used to locate the ligand binding sites on the protein molecule. The SiteMaps are helpful for finding the ligand that can interact with amino acid residues of the target. Grid generation is one of the crucial steps that determine the complexity of ligands that can be docked with targets for efficient interaction. The larger the binding sites the better will be the grid generated for docking.

2.5. Ligand Preparation

Compounds were prepared with the help of LigPrep (2.3) module [19], the drawn ligands were geometrically optimised by using the optimised potentials for liquid simulations-2005 (OPLS-2005) force field with the steepest descent followed by truncated Newton Conjugate gradient protocol. Partial atomic charges were computed using the OPLS-2005 force field. The LigPrep is a utility in Schrodinger software suite that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers, steric isomers and geometry minimization of ligands. Ligands were prepared using this utility in the software.

2.6. Molecular docking

Molecular docking tool was used to perform rigid flexible docking for predicting ligand binding attraction, efficiency and inhibitory constant. Two precision methods were used namely standard and extra precision methods within this tool. Our study used the extra precision (XP) method to dock the bioactive ligands. Here, only the active compounds that possessed the ligand binding efficiency were chosen to avoid penalties and to obtain favourable scores for accurate hydrophobic contact between the selected protein and the ligand. After getting the docking value the lead molecules that had the ligand to protein binding affinity were tested for other properties including electrostatic energy, Hydrogen bond side chain and back chain interaction, Pi-Pi stacking and Salt Bridge interaction. Glide is commercial software used for docking and to predict the binding to those active sites of pro-

teins in the proteome of the dengue virus which was used to identify the binding pockets and cavities in E and NS proteins.

3. RESULTS

The recognition and affinity of ligands towards antigens, E and NS proteins were interpreted from the inter-atomic distances and hydrogen bonding formed between the amino acid residues of docked protein-ligand complex structure. The prominent binding pockets and cavities in E and NS proteins were identified by using Glide module. Docking of E and NS proteins with selected 12 bioactive ligands from various medicinal plants belonging to different families of angiosperms (Table 1) were carried out and the docking scores and interaction characteristics were determined and the results are tabulated as shown in Table.2.

3.1. Docking interaction with E proteins:

Out of 12 ligands, Andrographolide showed the highest Glide score of -7.620 with 2 hydrogen bonds formed between the ligand and the amino acid molecules (Fig.2 & Table.3). Quercetin and Pinostrobin also showed the higher docking scores of -7.256 and -7.103 respectively. During docking, three hydrogen bonds formed between Quercetin molecule and amino acid residues of the E protein showed a perfect binding. Pinostrobin formed only one hydrogen bond between the ligand and the receptor. Among the 12 bioactive compounds, Andrographolide was identified to be the most suitable and/or potent drug for the DENV-1 serotype. However, Quercetin and Pinostrobin could also be used for dengue disease caused by DENV-1 with moderate potency.

The interaction of DENV-1 E-protein amino acid sequence with Andrographolide at an inter-atomic distance less than 5 Å showed that the interactions between the protein and ligand had occurred only in the active site pockets. The pocket of the active site was surrounded by 17 amino acids of which 13 of them were hydrophobic. The high affinity of the E-protein towards Andrographolide was favoured by two hydrogen bonds, formed by Val-130 and Met-196 with ligand molecule Andrographolide. The distance between the H-bonds of the above amino acids and the ligand molecules was 1.42 and 2.19 Å, respectively. The docking study revealed that van der Waals forces play an important role in stabilising the protein-ligand complex. The van der Waals interaction and hydrogen bonding formed by the reactive amino acid residues of E-protein with the ligand molecule led to binding of E-protein and Andrographolide (Fig 3).

In docking between E protein of DENV-2 with 12 bioactive compounds, Chalcone, Quercetin and Hesperitin showed the highest binding scores -7.580, -7.337 and -7.011 respectively (Table.3). Among the three, Quercetin showed 4 hydrogen bonds during docking (Fig.3a) while Hesperitin had two hydrogen bonds and Chalcone did not show any hydrogen bonds. however, due to the van der Waals forces and electrostatic attraction, Chalcone showed the highest binding score for which this drug was identified as the best drug for the treatment of DENV-2 caused dengue infection.

When 12 bioactive compounds were docked with DENV-3, only one compound interacted with the active binding site of DENV-3 which was Chalcone that showed a docking score of -7.342 (Fig. 4 & Table. 3). The remainder of 11 compounds was not able to enter the binding pockets of the E-protein. Therefore, we state that Chalcone could be used for the treatment of dengue disease caused by DENV-3. Likewise, docking was carried out with 12 bioactive compounds and DENV-4 serotype and only six compounds were able to interact with the binding site of E-protein receptor. Among the 6 compounds, Chalcone showed the highest binding score of -7.052 due to high van der Waals forces and electrostatic attraction even though no hydrogen bonding was observed (Fig.5). On the other hand, Naringin, which showed 6 hydrogen bondings between the ligand and the E-protein, exhibited only -6.023 Glide score due to low van der Waals forces and electrostatic attraction. Alpinetin and Hesperitin, which showed Glide score of -5.964 and -5.550 respectively, showed two hydrogen bondings during docking. Therefore, Chalcone was found to be effective in neutralising DENV-4 related dengue infection.

The Chalcone ligand molecule which showed the highest docking score with DENV-2, DENV-3 and DENV-4 E-proteins, was also able to dock with DENV-1 E- protein. the docking score was -6.272. Here 14 amino acids residues, out of 17 were hydrophobic. In this docking, the score obtained was due to the van der Waals forces and likewise, in the docking of DENV2, DENV3, DENV-4 and NS-1 Chalcone showed maximum docking scores of 7.580, -7.342, -7.052 and -5.278 respectively. In the DENV2 E-protein, out of five ligand binding sites, site 4 (score 1.068), was identified as the major active site for docking. In the DENV-3 E protein, out of ligand binding sites, site 4 was identified as the major active site for docking. This active binding site was lined with 22 amino acids of which 20 of them were hydrophobic and 2 were polar and the protein-ligand interaction was through the van der Waals forces. In the E-protein of DENV-4 out of five ligand binding sites, site 4 was identified as the major active site for docking. This active binding site was lined with 19 amino acids of which 18 of them were hydrophobic and one was polar with protein-ligand interaction being mediated through the van der Waals forces. Similarly, the 3-D structure of the E-protein of the DENV3 and DENV4 showed the 4th site to be the major active site that scored 1.338 and 1.296, respectively. This active binding site was lined with 17 amino acids of which 13 of them were hydrophobic, 2 were charged negative, one was polar and another one was glycine. Collectively, our results showed that the high affinity of the E-protein towards Chalcone was due to the van der Waals forces interaction rather than through hydrogen bonding between the atoms of E-protein and Chalcone. Therefore, in this docking van der Waals forces play an important role in stabilising the protein-ligand complex which resulted in a higher docking score over other ligands.

3.2. Docking interaction with NS proteins:

Docking interaction of 12 bioactive compounds with NS-1 protein showed a higher Glide score for Chalcone -5.278, Pinostrobin -5.225, Hesperidin -5.145 and Daidzein -5.031 (Table.3). Single hydrogen bonding was formed between the ligand, Chalcone and the receptor. However, due to high van der Waals forces and electrostatic attraction, Chalcone was able to get a high binding score (Fig.6). Pinostrobin formed two hydrogen bonds with amino acid residues of the binding site and scored -5.225. Hesperidin was docked with NS-1 through three hydrogen bondings. However, Chalcone was identified as the best drug for the treatment for patients having NS-1 protein in the blood serum due to high binding score rendered by van der Waals forces and electrostatic attraction.

NS-1 protein of dengue virus template showed 2 ligand-target sites where the first site was the major active binding site for docking. The glide score of docking for the first binding site was 0.574 which was higher than that of the second one. The pocket of the active binding site was surrounded by 13 amino acid residues, out of which four were hydrophobic, four were polar, two were positively charged, two negatively charged molecules and one was a glycine with hydrogen bond interaction sharing one oxygen.

When the NS-3 protein was docked with 12 bioactive compounds, Glabranin, Naringin and Hesperitin showed hydrogen bonding and Glide scores more than -6. Glabranin, which showed a higher glide score of -6.619 (Table.3) over the latter two, formed single hydrogen bonding with NS-3 protein molecule (Fig.7). Naringin, which also showed relatively high Glide score -6.592 over Hesperitin had developed an effective docking with 5 hydrogen bonds in addition to high van der Waals forces and electrostatic attraction. On the other hand, Hesperitin which showed a score of -6.432, had four hydrogen bonds interacting with the amino acid residues of NS-3 molecule. Even though the above three molecules were identified as good drug candidates against dengue virus that produces NS-3 antigen in the bloodstream, Glabranin was identified as the suitable drug candidate against NS-3 proteins in the blood serum. The NS-3 molecule showed five binding sites of which the first one was identified as the active binding site. Out of 12 biological ligand molecules, Glabranin showed a high affinity towards NS-3. This active binding site was lined with 22 amino acids of which 7 of them were hydrophobic, seven were polar, 4 were negatively charged, 3 were positively charged and 3 were interacting through hydrogen bonding.

Twelve bioactive compounds, when docked with a NS-5 protein of the dengue virus, Naringin, Hesperidin, Hesperitin, Fisetin and Quercetin (n=5), showed a Glide score ranging from -6.513 to -6.955 (Table.3). Among them Naringin had developed 7 hydrogen bonding with a NS-5 molecule (Fig.8.a); Quercetin with 5 hydrogen bonds (Fig 8.b), Fisetin formed 4 hydrogen bonds (Fig.8.c) and Hesperidin and Hesperitin developed 3 hydrogen bonds. Among the above five bioactive compounds, Naringin was identified as the best bioactive compound to combat dengue virus that produces NS-5 protein during the diseases process.

The NS-5 molecule showed five binding sites of which the second site was identified to be the active binding site. Out of 12 biological ligand molecules, Naringin showed high affinity with NS-5 and further to identify this, the active binding site was lined up with 18 amino acids of which 10 of them were hydrophobic, 5 were polar, 2 were negatively charged, one was glycine and 7 were interacting through hydrogen bonding. The binding interaction between the NS5 and the ligand Naringin was very strong showing 7 hydrogen bonding and high-affinity van der Waals forces.

4. Discussion

Plants and plant-derived compounds remain an important source for the discovery and development of new antiviral drugs due to the lesser adverse effects and their easy accessibility in nature [20-23]. There are numerous reports on the antiviral activity of various phytochemicals against dengue viruses and these include various flavonoids [24-27]. Flavonoids are basically low molecular weight phenolic compounds found widely in different kinds of medicinal plants that are used in natural plants-based medicine widely practised in India. Different types of flavonoids are found in fruits, roots, nuts, seeds, barks, stems and flowers of plants. One such flavonoid is Quercetin which can be found in some foods and fruits such as green and black tea, apple, onion, citrus, tomato and few other plants [28, 29].

In this study, 12 bioactive compounds that included Andrographolide, Quercetin, Pinostrobin, Alpinetin, Hesperidin, Chalcone, Daidzein, Fisetin, Pinocembrin-7-methyl ether, Glabranin, Naringin and Hesperitin were subjected to the virtual screening to be ideal ligands for DENV serotypes through molecular docking. Our study, results revealed that the Daidzein activity against DENV-2 was not significant compared to Quercetin (SI = 1.03) due to its low binding activity and therefore, this compound was declared to be unsuitable for further research and development to serve as an anti-dengue drug. Similarly, Hesperetin, the other flavonoid evaluated in our study, did not show anti-dengue activity in any of the stages of dengue viral infection as well as in replication processes, and this is contrary to the previously reported study on the antiviral activity of Hesperetin [30]. Based on the results we obtained from our study, we decided that Hesperetin may not be a suitable candidate for the further investigations towards developing an anti-dengue drug.

Among the tested bioactive flavonoids, Chalcone alone was found to be able to interact with E proteins of all four dengue serotypes and all the NS1, NS3 and NS5 proteins involved in dengue fever. Chalcone, a flavonoid, isolated from *Bridelia ferruginea* belongs to the family Euphorbiaceae is known to possess antimicrobial, fungicidal, anti-neuroinflammatory and antibacterial properties [31-34]. Re-

cently, Chalcones were identified to possess antitumor and antiviral properties [35, 36]. In the present investigation, the role of Chalcone as an antiviral bioactive compound has been evaluated along with other 11 bioactive compounds. Even though Chalcone was able to interact with all the DENV serotypes and NS proteins other flavonoids are much more effective for individual DENV serotypes and NS proteins (Table 2).

Hesperetin is a flavonone and its glycoside form, hesperidin (water soluble) are found in various citrus fruits while Naringin, on the other hand, is a flavanone glycoside found abundantly in orange juice [37]. Antiviral activity of Naringin is reported against HSV-1 and HSV-2 but this finding remains controversial [38, 39]. Daidzein is an isoflavone found in soybeans and its antiviral activity against influenza viruses has been reported previously [40]. Currently, there is no published data on the possible anti-dengue virus activities of Quercetin, Hesperetin, naringin and Daidzein. Recent studies have shown that flavonoids, such as Glabranine and 7-O-Methyle Glabranine have exhibited significant antiviral activities against dengue virus. Pinostrobin was reported to inhibit DENV-2 NS2B/NS3 protease, an enzyme important for dengue virus replication [12].

Zandi et al [43] suggested that flavonoids including Quercetin and Fisetin, exhibited significant DENV replication inhibition properties while Daidzein, Hesperetin and naringin did not show any significant anti-DENV replication activities. The anti-DENV replication properties of Quercetin was demonstrated by Fong [41] here it Quercetin was shown to hinder intracellular DENV viral replication. However, Quercetin does not affect the DENV attachment and entry processes.

The protein-ligand interaction plays a significant role in determining the suitability of drugs and/or antibodies for the treatment. A variety of computational methods is available to identify the suitable drugs. One such method is docking of drug molecules with receptors. The energy value obtained through docking is used as a criterion for the selection of drugs, which involves the identification of lead molecules. The lead molecules are those with maximum interaction having high negative e-value [42]. Thus, the concept of protein-ligand docking helps in evolving effective bioactive drugs for DENV serotypes.

Therefore, the present study has exploited the molecular docking and hydrophobic interactions of bioactive compounds with receptor molecules (E and NS proteins) causing dengue fever to design and investigate the best candidate for treating dengue viral infections. In the present investigation, the ligand binding site showed the occurrence of more number of hydrophobic amino acid residues. As the hydrophobic amino acids have been enriched in most of the ligand binding sites, effective ligand- protein interactions were possible.
Hydrophobic interactions are the most important noncovalent forces that are responsible for the different phenomena such as structural stabilisation of proteins, binding of enzymes to substrates and folding of proteins [43]. The interaction of hydrophobic amino acids of antigen with the hydrophobic zones on the protein (antibody) surface is an important event in molecular docking [44]. The noncovalent bonds hold the ligand- protein complex together. These bonds are weak by nature and therefore, they have to work together to make a significant effect. In addition, the combined bond strength is greater than the sum of the individual bonds. Kunik and Ofran [45] pointed out the importance of more hydrophobic amino acid residues which are more important in the molecular docking interaction in which 50% of the attracting force is contributed by the hydrophobic amino acids in the ligand binding sites on the protein molecules. Hydrogen bonds stabilise the molecular interaction whereas other weak interactions such as van der Waals forces, hydrophobic interactions and electrostatic forces improve the binding specificity between ligand and proteins [46]. Chandra, Singh [47] also reported that hydrophobic amino acids are more frequent in a binding group than in the nonbinding group.

Extensive researches have been carried out in the development of vaccines against this infection but none of the efforts have been proved successful till today. There is a pressing need to design new and novel approaches for drug development to combat this viral infection [48]. Use of natural pharmaceuticals like medicinal plants may prove an efficient path for solving this unresolved medical problem [49] and flavonoids can play an important role in the cure of Dengue virus infections [50].

In the past few years, binding analyses of compounds through *in-silico* techniques prior to their lab manufacturing and examination has become a common and useful practice among researchers. One of the popular computational techniques, used in medical research, is the molecular docking which is used to find the binding behaviours of small molecules against their target proteins. This technique has been proved to be helpful in providing a clear molecular vision of viral genes and identification of novel inhibitory compounds against fatal viral infections [1].

Drug discovery is a time-consuming and expensive process, with a high failure rate and for these reasons new drug design approaches and methodologies keep constantly changing in order to reduce the time and costs towards drug development. In recent years, interest has increasingly been focused on computer-based techniques and molecular modelling to design and virtually evaluate novel potential drugs on the computer before the drugs being prepared and tested in a laboratory. *In silico* methodologies are suitable to tackle various issues, because of their flexibility and increasing reliability. The application of *in silico* technologies to antiviral research has already led to the design of several compounds approved by the US Food and Drug Administration and now are used in clinical therapy [51].

5. Conclusion

Molecular docking analysis of bioactive compounds as ligands with the target E and NS proteins showed that the bioactive compound Chalcone is the best candidate that has high binding score compared to other ligand molecules that can be employed for treating DENV. Our findings revealed that the bioactive molecule Chalcone has promising interaction with E proteins of all four dengue serotypes and NS1. On the other hand, Andrographolide, Quercetin Pinostrobin were found to have similar interactions with DENV 1 serotypes which indicated that these compounds may be used to combat DENV 1 serotype. While DENV 2 can be treated effectively with Chalcone or Quercetin based on their comparative docking scores, -7.580 and -70337 respectively. However, dengue infection caused by DENV 3, can only be treated with Chalcone as it is the only compound that has the ability to be docked with DENV 3 E proteins. Likewise, DENV 4 related dengue infection can be controlled by Chalcone as it has a high score of -7.052. Furthermore, for patients that test positive for NS1 proteins, Chalcone will be a potential bioactive compound to neutralise the NS1 proteins while Glabranin and Narigin will be effective to neutralise NS 3 and NS 5 proteins, respectively. In conclusion, Chalcone was found to be a potential drug candidate that can be subjected to further investigations towards the development of anti-dengue viral drug and/or towards anti-dengue therapeutic purposes.

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Conflict of interest

We declare that we have no conflict of interest.

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Tables

Table. 1. Source and Bioactivity of Phyto-compounds against Envelope and Non-structural proteins.

S. No.	Name of the Phytocompounds	Medicinal plant species	Family	Habit	Parts	Type of serotypes treated
1.	Andrographolide	*Andrographis paniculata (Burm.f) Nees*	Acanthaceae	Herb	Leaves	DENV 1
2.	Quercetin	*Phyllanthus emblica*	Euphorbiaceae	Tree	Fruit	DENV1-4
3.	Pinostrobin	*L. Boesenbergia pandurat. Schult.*	Zingiberaceae	Tree	Leaves	DENV 2& NS3
4.	Alpinetin	*Boesenbergia pandurat. Schult.*	Zingiberaceae	Tree	Leaves	DENV 2& NS3
5.	Hesperidin & Hesperitin	*Citrus unshiu* Marc.	Rutaceae	Small tree	Fruit	DENV-2
6.	Chalcone	*Bridelia ferruginea* Benth. & *Tephrosia purpurea* L.	Euphorbiaceae	Tree	Leaves	DENV1-4
7.	Daidzein	*Momordica dioica* Roxb. ex Willd.	Cucurbitaceae	Shrub	Seed	DENV1-4
8.	Fisetin	*Capsella bursa-pastoris* L.	Brassicaceae	Herb	Leaves	DENV-2
9.	Pinocembrin-7-methyl ether	*Camellia sinensis* (L.) O. Kuntze	Theaceae	Small tree	Leaves	DENV-1
10.	Glabranin	*Melicope glabra* Blume	Rutaceae	Tree	Leaves	DENV-2
11.	Naringin	*Citrus aurantifoli. Swingle.*	Rutaceae	Climber	Fruit	DENV-2

Table. 2. Results of molecular docking (Glide) of bioactive compounds with E and NS proteins

Bioactive compounds	DENV-1	DENV-2	DENV-3	DENV-4	NS-1	NS-3	NS-5
Andrographolide	**-7.62**	-5.034	No binding	No binding	-4.527	-5.45	-6.047
Quercetin	-7.256	**-7.58**	No binding	No binding	-4.541	-5.97	-6.513
Pinostrobin	-7.103	-7.036	No binding	No binding	-5.225	-6.223	-6.15
Alpinetin	-6.865	-6.837	No binding	-5.964	-4.637	-6.087	-5.297
Hesperidin	-6.826	-5.969	No binding	No binding	-5.145	-6.171	-6.726
Chalcone	-6.272	**-7.333**	**-7.342**	**-7.052**	**-5.278**	-5.361	-5.867
Daidzein	-6.631	-6.425	No binding	No binding	-5.031	-6.288	-6.163
Fisetin	-6.575	-7.287	No binding	No binding	-4.767	-5.969	-6.682
Pinocembrin-7-methyl ether	-6.498	-6.338	No binding	-5.038	-4.4	-5.736	-5.994
Glabranin	-6.086	-3.35	No binding	-5.795	-4.097	**-6.619**	-6.407
Naringin	-6.035	-5.906	No binding	-6.023	-3.44	-6.592	**-6.955**
Hesperitin	-5.625	-7.011	No binding	-5.55	-3.274	-6.432	-6.643
NIT008	**-7.373**	-5.564	No binding	No binding	-5.386	-5.863	-6.128

Table. 3. Molecular characteristics of docking between bioactive compounds and E and NS proteins of DENV

S.No	Drugs and Characters	DENV1	DENV2	DENV3	DENV4	NS1	NS3	NS5
1	**Andrographolide**							
	1.H-Bond	2	1			4	3	2
	2.Wander Val forces			No binding	No binding			
	3.Electrostatic forces							
	4.Dotted contour	Incomplete	Incomplete			Incomplete	Incomplete	Incomplete
	5.Position of ligand	Center	Entrance			Peripheral	Center	Center
	6.Solvent-exposed surface area	3	1			8	5	6
2	**Quercetin**					2	3	5
	1.H-Bond	3	4					
	2.Wander Val forces			No binding	No binding			
	3.Electrostatic forces							
	4.Dotted contour	Incomplete	Incomplete			Incomplete	Incomplete	Incomplete
	5.Position of ligand	Center	Center			Peripheral	Center	Peripheral
	6.Solvent-exposed surface area	6	3			13	7	14
3	**Pinostrobin**							
	1.H-Bond	1	1			1	1	3
	2.Wander Val forces			No binding	No binding			
	3.Electrostatic forces							

	4.Dotted contour	Complete	Incomplete			Incomplete	Complete	Incomplete
	5.Position of ligand	Center	Center			Peripheral	Center	center
	6.Solvent-exposed surface area	Nil	1			9	2	12
4	**Alpinetin**							
	1.H-Bond	1	1		2	3	1	Nil
	2.Wander Val forces			No binding				
	3.Electrostatic forces							
	4.Dotted contour	Incomplete	Incomplete		Incomplete	Incomplete	Complete	Incomplete
	5.Position of ligand	Center	Center		Center	Peripheral	Center	Center
	6.Solvent-exposed surface area	4	1		1	12	1	10
5	**Hesperidin**							
	1.H-Bond	3	6			3	4	3
	2.Wander Val forces			No binding	No binding			
	3.Electrostatic forces							
	4.Dotted contour	Incomplete	Incomplete			Incomplete	Incomplete	Incomplete
	5.Position of ligand	Entrance	Peripheral			Peripheral	Center	Center
	6.Solvent-exposed surface area	14	22			24	4	12
6	**Chalcone**							
	1.H-Bond	Nil	Nil	Nil	Nil	1	Nil	Nil
	2.Wander Val forces							
	3.Electrostatic							

	forces							
	4.Dotted contour	Complete	Complete	Complete	Complete	Incomplete	Incomplete	Incomplete
	5.Position of ligand	Center	Center	Center	Center	Peripheral	Peripheral	Center
	6.Solvent-exposed surface area	2	Nil	Nil	Nil	9	3	4
7	**Daidzein**							
	1.H-Bond	1	2			2	2	1
	2.Wander Val forces			No binding	No binding			
	3.Electrostatic							
	forces							
	4.Dotted contour	Incomplete	Incomplete			Incomplete	Complete	Incomplete
	5.Position of ligand	Center	Entrance			Peripheral	Center	Peripheral
	6.Solvent-exposed surface area	5	5			15	1	11
8	**Fisetin**							
	1.H-Bond	3	4			2	4	4
	2.Wander Val forces							
	3.Electrostatic			No binding	No binding			
	forces							
	4.Dotted contour	Incomplete	Incomplete			Incomplete	Incomplete	Incomplete
	5.Position of ligand	Entrance	Entrance			Peripheral	Center	Peripheral
	6.Solvent-exposed surface area	4	5			12	3	14
9	**Pinocembrin-7-methyl ether**							
	1.H-Bond	1	Nil		1	1	1	4
	2.Wander Val forces			No binding				

	3.Electrostatic forces						
	4.Dotted contour	Complete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
	5.Position of ligand	Center	Entrance	Center	Peripheral	Center	Peripheral
	6.Solvent-exposed surface area	Nil	5	1	9	4	12
10	**Glabranin**						
	1.H-Bond	1	1	1	Nil	1	3
	2.Wander Val forces						
	3.Electrostatic forces		No binding				Incomplete
	4.Dotted contour	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Center
	5.Position of ligand	Center	Outside	Center	Peripheral	Center	14
	6.Solvent-exposed surface area	5	14	3	12	2	
11	**Naringin**						
	1.H-Bond	2	3	6	1	5	7
	2.Wander Val forces						
	3.Electrostatic forces		No binding				
	4.Dotted contour	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
	5.Position of ligand	Entrance	Peripheral	Peripheral	Peripheral	Center	Center
	6.Solvent-exposed surface area	17	14	11	18	6	14
12	**Hesperitin**						
	1.H-Bond	3	2	2	2	4	3

2.Wander Val forces							
3.Electrostatic forces							
4.Dotted contour	Incomplete	Incomplete	No binding	Complete	Incomplete	Incomplete	Incomplete
5.Position of ligand	Entrance	Peripheral		Center	Peripheral	Center	Peripheral
6.Solvent-exposed surface area	15	6		Nil	11	4	11

Figures

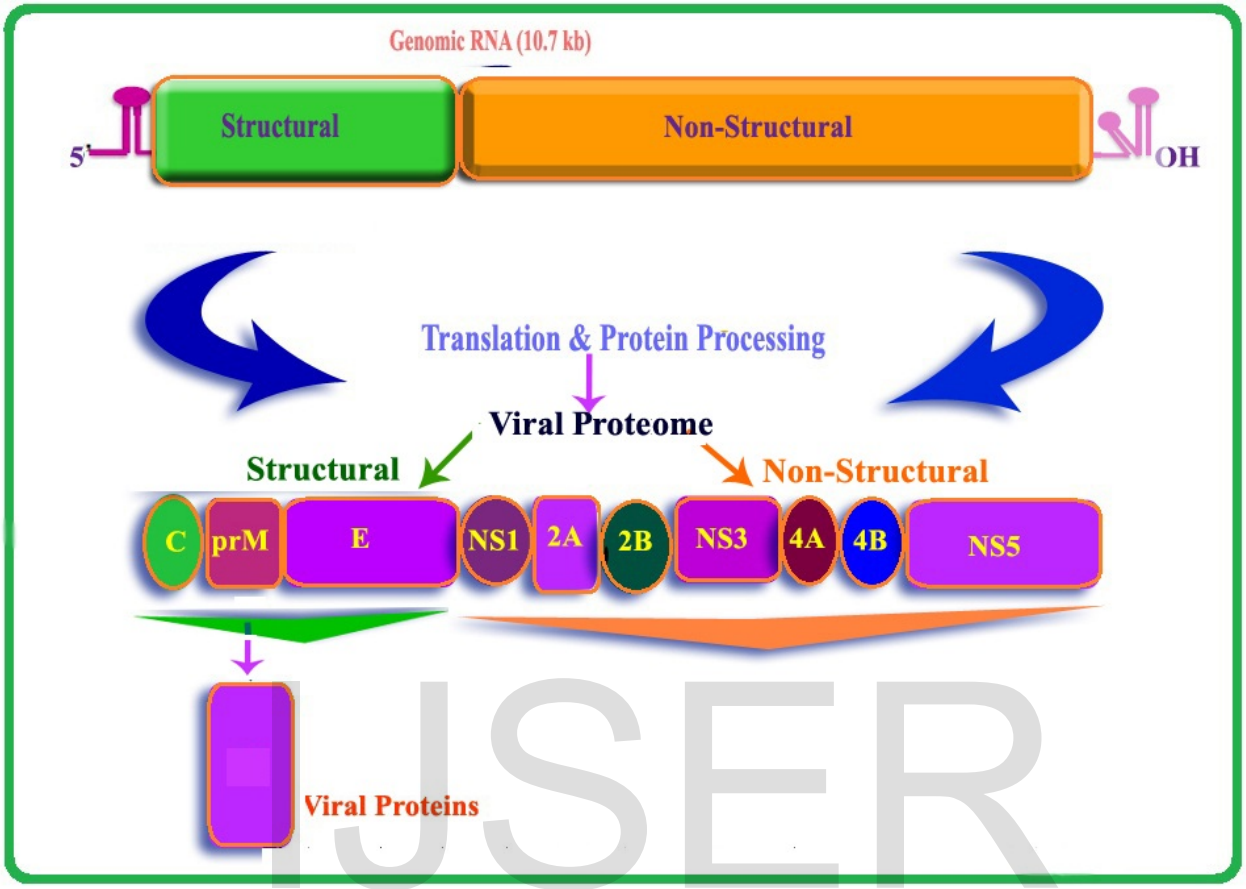

Fig 1. Flavivirus RNA and its translation into proteins involved in the Dengue life cycle. (Modified from Lim, Rahman [1]. The plus strand enveloped dengue virus (*Flavi virus* genus) belongs to *Flaviviridae* family contains a 10.7 kb single strand RNA. The single strand RNA that has been translated into a single polyprotein which processed into 10 mature proteins by co-translational cleavage and the 10 mature proteins consist of three structural proteins (capsid (c), pre-membrane (prM), envelope (E)) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [1]

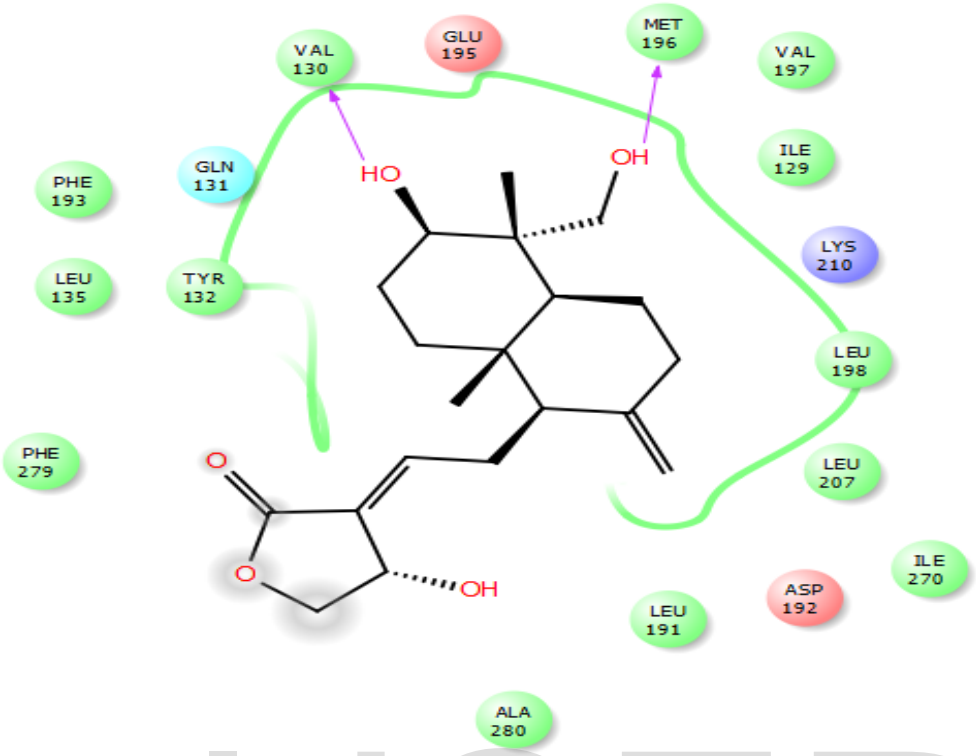

Fig.2. Molecular docking of bioactive ligand, Andrographolide with the active binding site of DENV1 E-protein. Charged (Negative), Charged (positive), Polar, Hydrophobic and Glycine

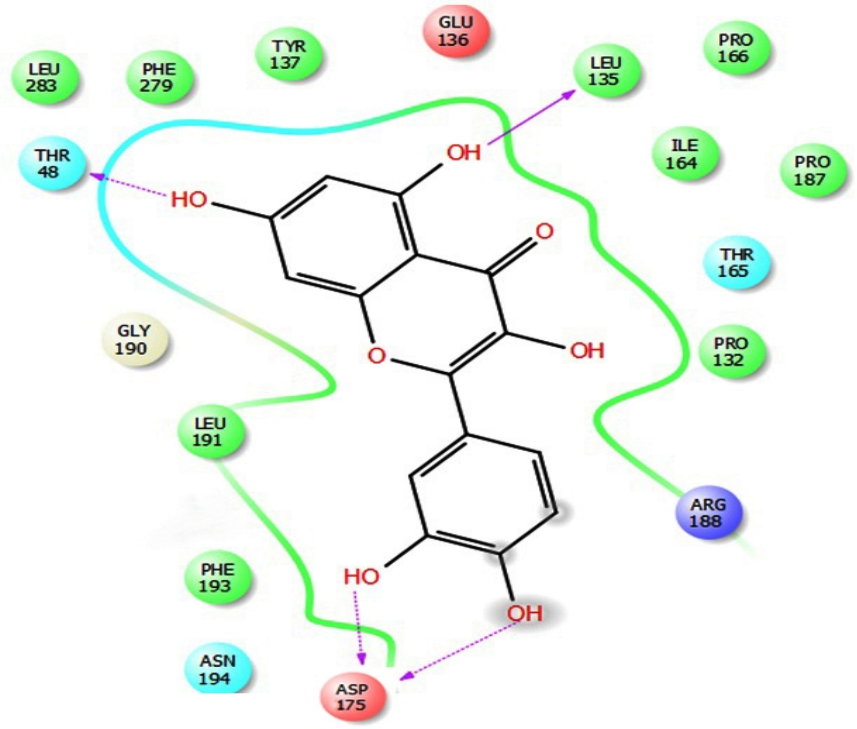

Fig.3. Molecular docking of bioactive ligand, Quercetin with the active binding site of DENV2 E-protein

Charged (Negative), Charged (positive), Polar, Hydrophobic and Glycine

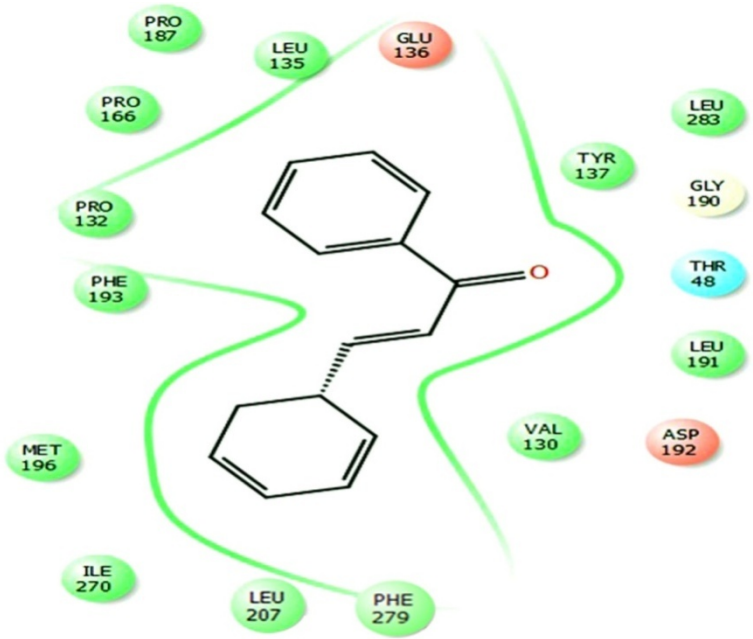

Fig.4. Molecular docking of bioactive ligand, Chalcone with active binding site of DENV3 E-protein

Charged (Negative), Charged (positive), Polar, Hydrophobic and Glycine

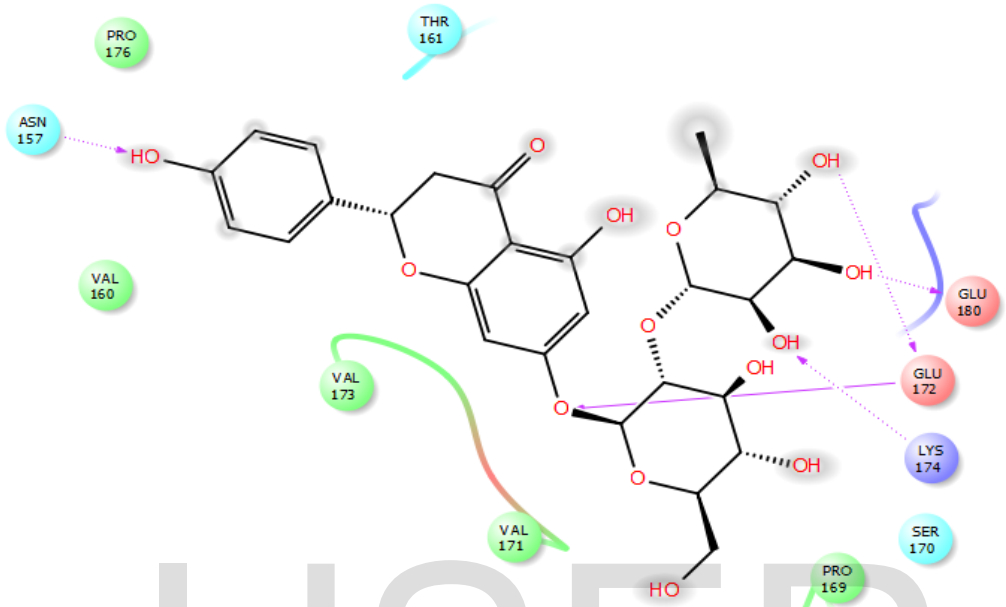

Fig.5. Molecular docking of bioactive ligand, Naringin with active binding site of DENV4 E-protein

Charged (Negative), Charged (positive), Polar, Hydrophobic and Glycine

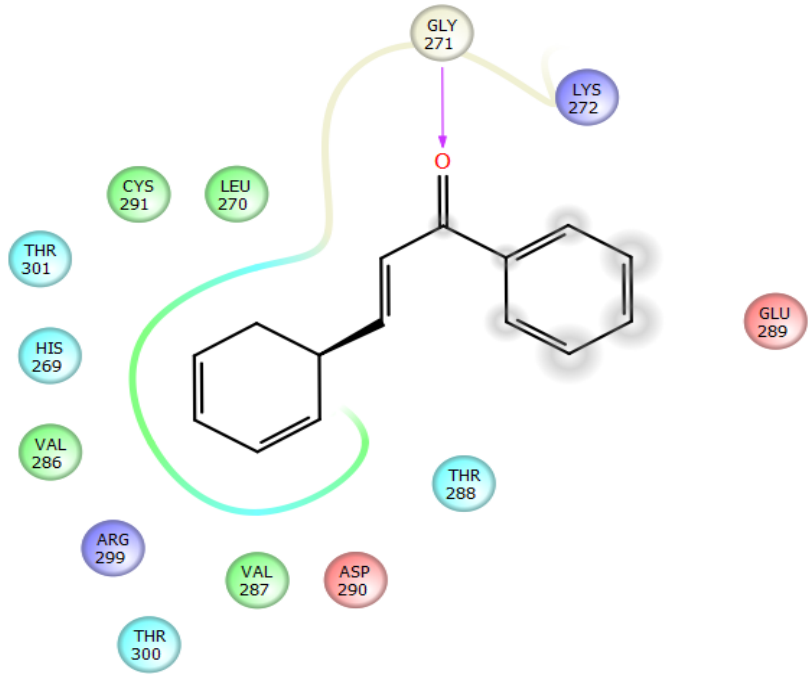

Fig.6. Molecular docking of bioactive ligand, Chalcone with active binding site of DENV NS1-protein

Charged (Negative), Charged (positive), Polar, Hydrophobic and Glycine

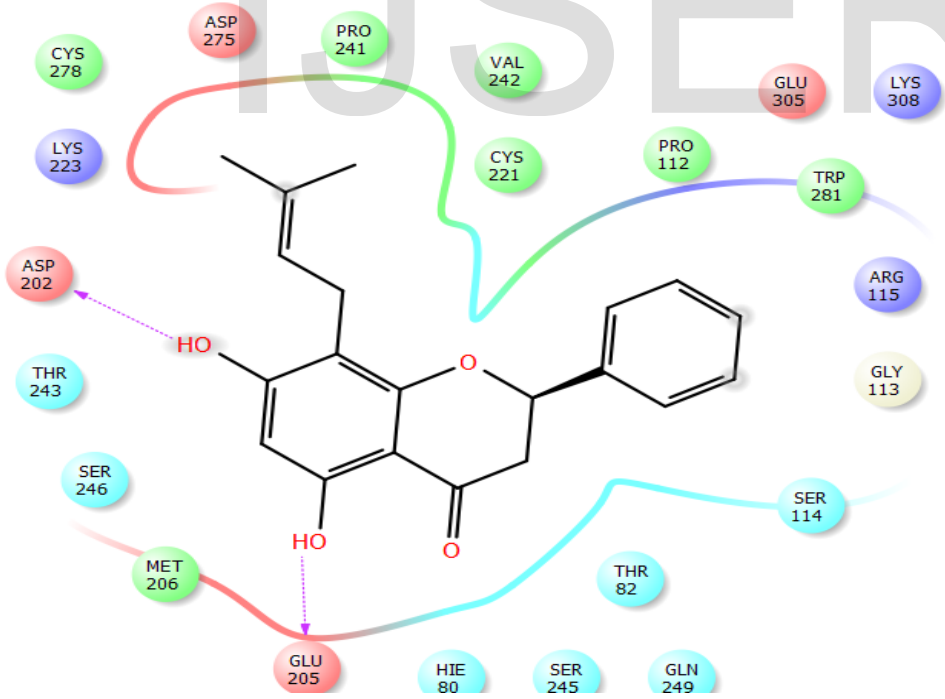

Fig.7. Molecular docking of bioactive ligand, Glabranin with the active binding site of DENV NS3-protein.

Charged (Negative), Charged (positive), Polar, Hydrophobic and Glycine

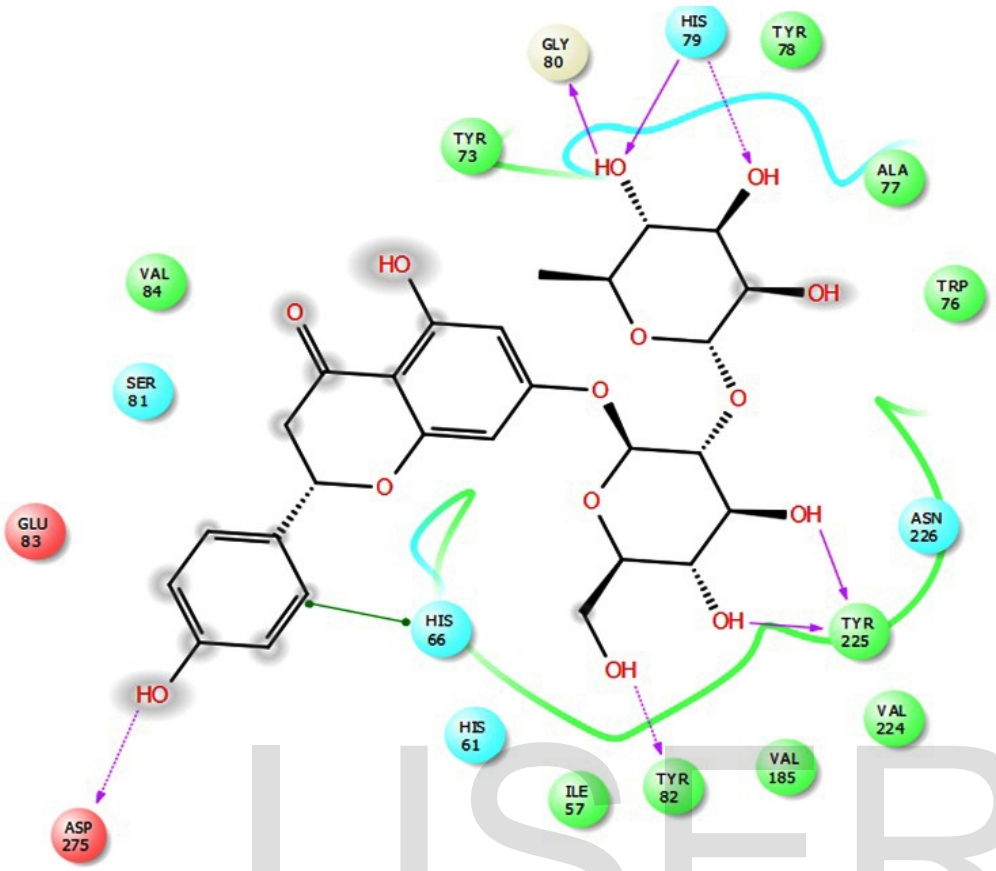

Fig.8. Molecular docking of bioactive ligand, Naringin with active binding site of DENV NS5-protein

Charged (Negative), Charged (positive), Polar, Hydrophobic and Glycine