Pak. J. Bot., 53(1): 309-319, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-1(17)

IN SILICO EXPLOITATION OF ANTIVIRAL PHYTOCHEMICALS AGAINST DENGUE

FAISAL NOUROZ[1,2], MADIHA MEHBOOB[1], TIBGHA MOBIN[3] AND SAJID KHAN[1]

[1]*Department of Bioinformatics, Hazara University Mansehra, Pakistan*
[2]*Department of Botany, Hazara University Mansehra, Pakistan*
[3]*Department of Biotechnology, COMSATS University Islamabad, Abbottabad Campus, Pakistan*
**Corresponding author's email: faisalnouroz@gmail.com*

Abstract

Phytochemicals are plants natural compounds, ethno-pharmacologically used as anti-hepatitis, anti-cancer, anti-diabetic, anti-fungal and anti-viral agents. Computer-aided drug designing (CADD) is flourishing technique nowadays and various natural plant products or phytochemicals are exploited to design potential drugs against various diseases. Dengue fever is caused by the dengue virus (DANV) and is a tropical mosquito-borne infectious disease. DANV infections are among the most important mosquito-infected diseases that are widespread in subtropical and tropical countries (>100). Currently, the dengue fever is becoming a serious health risk. There is need of treatment in contrary to the growing issues of dengue fever and the presence of resistant mutants of DANV in the world. In present research, potential and beneficial anti-dengue drug from plants natural compounds is proposed using CADD, which requires the utilization of information (Biochemical) of ligand-receptor interaction in turn to assume drug treatments. To find biochemical information, docking interactions of selected ligands with E-glycoprotein (4UT6) active site was done which showed effective results against the DANV. Ligand-receptor docking were interpreted by different interactions such as hydrogen bonding, ionic and hydrophobic interactions. Based on the interaction assessment and binding energies of the compounds, one was considered as a "lead compound". Six compounds were placed with in the active site of E-glycoprotein (PDB id: 4UT6). Ligands interaction with the active site of E-glycoprotein was assessed. On the bases of high binding interactions and activity, the assessed drug compounds will be proposed in the laboratory as a future plan for clinical testing and synthesis against Dengue virus.

Key words: *In-silico*, Drug, Dengue, E-glycoprotein, Ligand, Molecular docking

Introduction

Phytochemicals are the natural products derived from plants and have been recognized as an important, effective and beneficial alternative to manage various infections or diseases such as dengue (Lee *et al*., 2013). Medicinal plants as herbal or natural products have been used as a source of treatment for a variety of diseases due to traditional healing (Okonkwo, 2012), complex bioactive substances and rich drug sources (Agnaniet *et al*., 2016). Several plants have been recently listed for their ethno-pharmacological uses especially treating the most important diseases such as hepatitis, cancer, diabetes and dengue fever. Numerous compounds from medicinal plants such as *Kalanchoe gracilis, Glycyrrhiza uralensis, Curcuma longa*, *Azidarachta indica* with anti-viral activities have been recently reported (Wang *et al*., 2016). Natural products are less toxic and cost effective in comparison to synthetic drugs (Babar *et al*., 2013). Current advances in computational biology methods have expanded the possibility of working in the field of drug development. It is now believed that molecular docking is the main method for predicting the predominant binding mode of a ligand with a defined 3D protein structure (Kiat *et al*., 2006) in drug design and screening of antiviral compounds discovered against scary diseases.

Dengue virus (DENV) is a single stranded RNA virus of family Flaviviridae, genus Flavivirus and is the main causative agent of Dengue (feverish disease). Flaviviruses are transmitted during a blood meal by mosquitoes bite (*Aedes albopictus* or *Aedes aegypti)*. The four serotypes of dengue are DEN-1, DEN-2, DEN-3 and DEN-4, which cause dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). The dengue fever is prevalent mainly in the tropical and subtropical regions and more than 100 countries are affected with an estimation of 390 million infections worldwide per annum, of which 96 million were clinically diagnosed (Bhatt *et al*., 2013). The developing of serious dengue infection is mostly observed among individuals, who are infected by a different type of DENV from first time infection. This is called second or heterotypic infection. For this reason, an acute disease first arises amongst the persons in zones wherever more than one serotype travels simultaneously. Infection with Dengue virus provides long-life immunity against the disease resulting from that particular type (reinfection), which promotes the feasibility of developing a vaccine, and shows effective results against Dengue. However, the infection only provides short-term immunity to the other three types of Dengue virus (Mizumoto *et al*., 2014).

According to World Health Organization (WHO), DENV is single stranded RNA virus causing Dengue, a mosquito-borne disease that affects both children and adults. Symptoms may appear three to fourteen days after infective mosquito bite. It is not directly transmitted to humans and requires a vector (mosquito) for its transmission. The symptoms are mild to high fever, acute headache, pain in the back of eyes, severe joint pain, muscle pain and heavy rashes. There is currently no WHO recommendation for the use of any specific drug for dengue fever or any effective treatment. Persons with dengue are advised to get plenty of rest, consume plenty of fluids, and relieve fever using paracetamol under medical advice (Anon., 2016). It is often needed to combat dengue infection in safe, low cost and effective drugs with inhibitory activity against DENV, particularly in the most affected countries with limited resources (Rothan *et al*., 2014).

In-silico drug designing is a complete and useful technique to design new drugs using information about biochemical activities of biological processes, chemical compounds. In the current era, the bioinformatics tools and softwares are greatly facilitating the discovery of *in-silico* drugs. In the current study, anti-viral plants were selected for screening potential anti-dengue compounds using *in-silico* approaches. The envelop protein (E-glycoprotein) of DENV2 was chosen as the drug target against dengue. The objective of the study was the *in-silico* investigation of phytochemicals exhibiting potential anti-viral activities against DENV infection.

Materials and Methods

Template selection and optimization: The experimentally designed 3D (crystal) structure of DANV-2 E-glycoprotein (PDB id: 4UT6) was recovered from Protein Data Bank (PDB) for current study. The structural E-glycoprotein was selected as target due to the presence of immune-dominance of the fusion loop epitope (FLE), a segment of the E-glycoprotein buried at the interface of the E dimers coating mature viral particles making it more virulent. After the target is defined; the candidate drug targets were chosen by the analysis of formerly acknowledged drugs with powerful anti-dengue effects or by designing new inhibitory compounds. The overall dengue drug designing pathway is represented in (Fig. 1). Initially, more than a hundred plants having anti-viral compounds were chosen. Subsequently, 40 potential plants with anti-viral phytochemicals were short-listed. Than all those phytochemical ligands that violate the Lipinski's rule of five were rejected and best phytochemicals were selected for further analysis.

Screening of Anti-dengue inhibitors: A number of E-glycoprotein inhibitors were identified and selected for *in-silico* drug designing and molecular docking studies. Six compounds were selected for docking analysis with the drug like properties against DANV. These six compounds were selected by assessing their efficacy and efficiency against viruses based on recent investigations on DANV. Selected drug inhibitors were retrieved by applying a series of filters such as FDA approved drug, toxicity class, structural organization, ADMET properties, control and drug screening. Drug scanning to control the satisfactory conditions of the compound as a drug candidate was performed by using ADMETSAR (Cheng *et al*., 2012) to ensure that the compounds retained the appropriate molecular properties, being a candidate drug (Table 1).

Molecular docking: The selected six anti-dengue agents were taken from PubChem (http://www.ncbi.nlm.nih.gov/pccompound) and ZINC databases (Irwin *et al*., 2012). Ligands docking such as inhibitors and the recommended drugs with the E-glycoprotein receptor (4UT6) structure were performed by utilizing AutoDock (4.2) (Morries *et al*., 2009). AutoDock gives the ligand structure and the binding interactions of the six chosen inhibitors along with the target active site of proteins. Addition of atoms of hydrogen, removal of water molecules were done before the molecular docking. For ligand preparation, after entering the ligand, the Gasteiger charges were combined with non-polar hydrogen ligands. Root detection was selected in the majority of atoms of the ligand and creating rotatable or non-rotatable bonds. Using the autogrid and 0.547 Å spacing, the 90x90x90 grid box was utilized to cover the whole molecule (receptor).

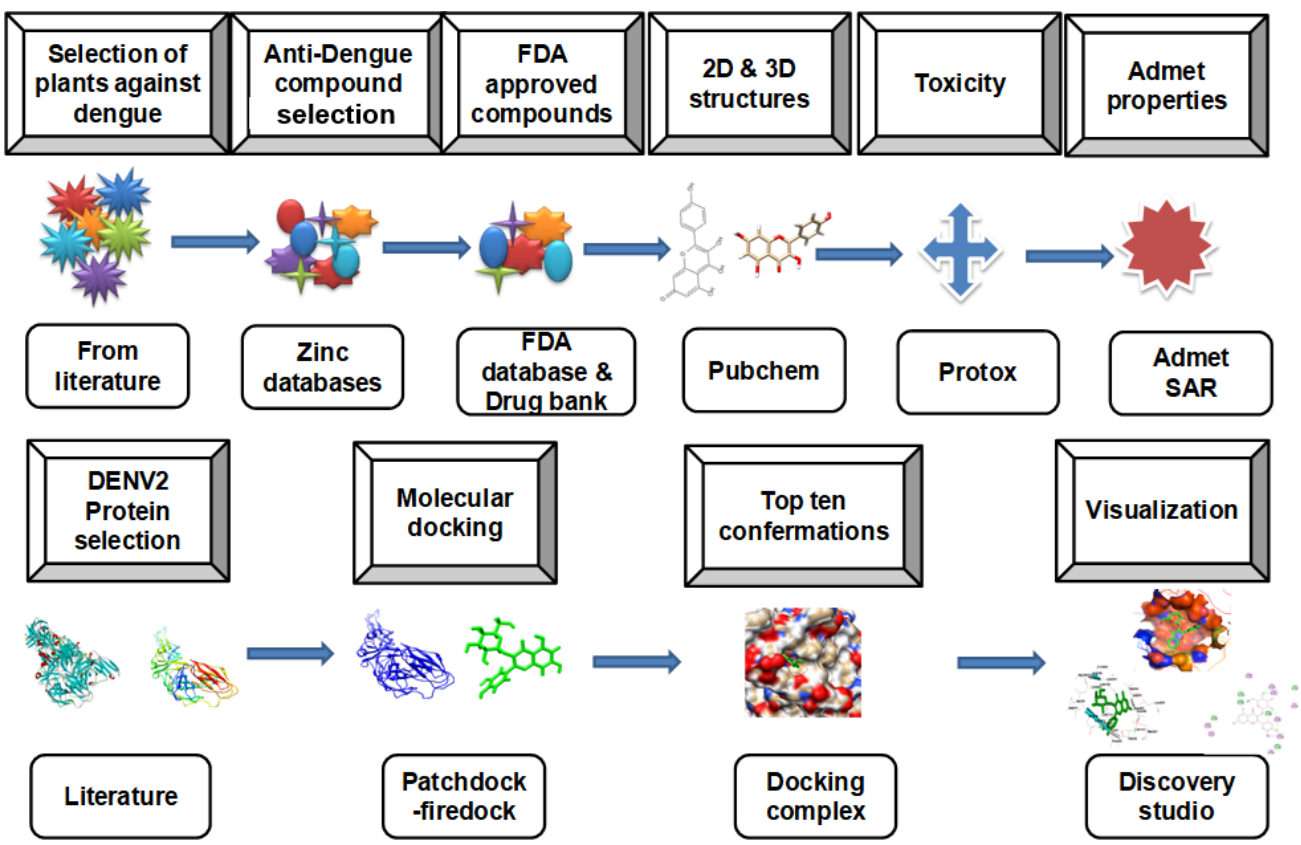

Fig. 1. Schematic representation of methodology used in current study for drug designing against DENV.

Table 1. Admet properties of the anti-dengue compounds.

Properties	ADMET properties	Hyperoside	Cissampeloflavone	Ethylene glycol	Eugenol	Phenol	Apigenin
Absorption	Blood-brain barrier	BBB+	BBB+	BBB+	BBB+	BBB+	BBB+
	Human intestinal absorption	HIA+	HIA+	HIA+	HIA+	HIA+	HIA+
	Aqueous solubility (LogS)	-3.2198	-3.5343	1.2264	-3.7543	-0.1541	-2.7765
	Caco-2 permeability (LogPapp)	Caco-2+	Caco-2+	Caco-2+	Caco-2+	Caco-2+	Caco-2+
	P-glycoprotein inhibitor	0.9552 0.9352	0.7135 0.7968	0.9503 0.9538	0.7379 0.5145	0.9790 0.9917	0.9543 0.7525
Metabolism	CYP450 1A2 inhibitor	0.8896	0.9501	0.8432	0.9644	0.6114	0.9222
	CYP450 2C9 inhibitor	0.8024	0.8448	0.9267	0.5201	0.9654	0.7746
	CYP450 2D6 inhibitor	0.5844	0.9302	0.9729	0.9372	0.9746	0.9231
	CYP450 2C19 inhibitor	0.6248	0.9606	0.9355	0.8993	0.8981	0.7043
	CYP450 3A4 inhibitor	0.5153	0.8210	0.9657	0.6321	0.9523	0.9580
Toxicity	AMES toxicity	0.6384	0.6571	0.9133	0.7310	0.9454	0.8906
	Carcinogens	0.6006	0.5690	0.9634	0.5396	0.8695	0.7316
	Biodegradation	BD+	BD+	BD+	BD+	BD+	BD+
	Acute oral toxicity	NT	NT	NT	NT	NT	NT
	Carcinogenicity	NC	NC	NC	NC	NC	NC

Non-toxic; NC – Non-carcinogen

The docking parameter file for rigid docking is therefore made by identifying the molecule that is rigid after this specification of the ligand was done. Ligand-receptor docking was constructed using Patch Dock (Schneidman-Duhovny *et al*., 2005). This method of docking was performed for six ligands having a receptor that has ten different conformations. These 10 different conformations were entered into Fire Dock for result refinement. Following, the molecular docking; protein-ligand complex was assessed to attain all the conformations. Out of these conformations, those with less binding energy value were chosen as the protein-ligand complex, which were recorded in PDB format. Once the molecular docking was completed, the protein-ligand complexes were closely analyzed using Discovery Studio (DS) software (Qamar *et al*., 2014) and then ligand-receptor interactions were investigated. Ligand-receptor interactions containing hydrogen bonds, ionic bonds, and covalent bonds within a pocket were examined utilizing the Discovery Studio. This scheme is specified just for one ligand in molecular docking. For this reason, the docking of all the compounds to the protein molecule was done to capture each compound's complex and then examined in DS. Geometry optimization was done in Chimera 1.6.1 (https://www.cgl.ucsf.edu/chimera/olddownload.html). The whole methodology is summarized in Fig. 1.

Results

Molecular docking: The docking process is initiated usually by binding of smaller molecule to a larger molecule like protein or enzymes. In the current study, initially above hundred plants with anti-viral compounds were chosen. Subsequently, 40 plants were short-listed with 200 anti-microbial phytochemicals, of which 6 most common anti-dengue compounds were selected. All those phytochemical ligands that violate Lipinski's rule of five were rejected. The six common anti-dengue phytochemicals were hyperoside, cissampeloflavone, ethylene glycol, eugenol, phenol and apigenin. Based on the binding of drugs (anti-dengue) to the E-glycoprotein (4UT6) (receptor protein), active sites were sought. Following this experiment, based on the x-score and lowermost binding energy, the best ligand-protein conformation was generated and selected to examine the binding orientation and conformations within E-glycoprotein binding pocket. The amino acids contained by 5Å (i.e. active site) were acknowledged. According to this study the major factors of binding pockets were the residues GLY349, PHE337, GLU338 and LEU351. The active site and hydrophobic pocket of E-glycoprotein along with selected inhibitor molecules for all the six compounds is displayed in (Fig. 2). Protein-ligand interactions (docked complexes) showed that all bioactive compounds were fully buried within the binding pocket of E-glycoprotein (Fig. 3). The dockings were then performed utilizing the E-glycoprotein target protein in the test set compounds. After performing the docking of all the compounds with E-glycoprotein receptor protein, the Discovery Studio was used to visualize these docked complexes. The best docking complex was observed with hyperoside, while other compounds (cissampeloflavone, ethylene glycol, eugenol, phenol and apigenin) also showed efficient docking results. Then QSAR descriptors were used for the analysis of interaction of complexes such as hydrophobic interactions, ionic interaction and hydrogen bonds. The 2D structural analysis showed hydrophobic and ionic interactions of hyperoside with E-glycoprotein receptor protein.

Lead identification: Based on the binding interactions of six compounds, a "lead compound" was detected. The compounds hyperoside, cissampeloflavone, ethylene glycol, eugenol, phenol and apigenin displayed considerable interactions within the active site (5Å) of E-glycoprotein amino acids. The binding interaction of these showed that hyperoside was the lead compound forming five hydrogen bonds with the amino acids GLY349, PHE337, PHE367, GLU338 and LEU351 (Fig. 4) within the active site of E-glycoprotein with the distance of 2.96Å, 2.08Å, 2.36Å, 3.03Å, 3.06Å. Carbon bonds are formed by hyperoside with the E-glycoprotein receptor protein. The 3D crystal structure of E-glycoprotein showed residues close to the ligand molecule (Fig. 5). The medicinal activities of selected plants was identified, where majority of plants have shown the inhibitory activities against the DENV indicating potential control against dengue. Maximum activities were shown against Dengue, while anti-viral, anti-inflammatory, anti-cancer and antibacterial activities were also detected in these plants (Fig. 6).

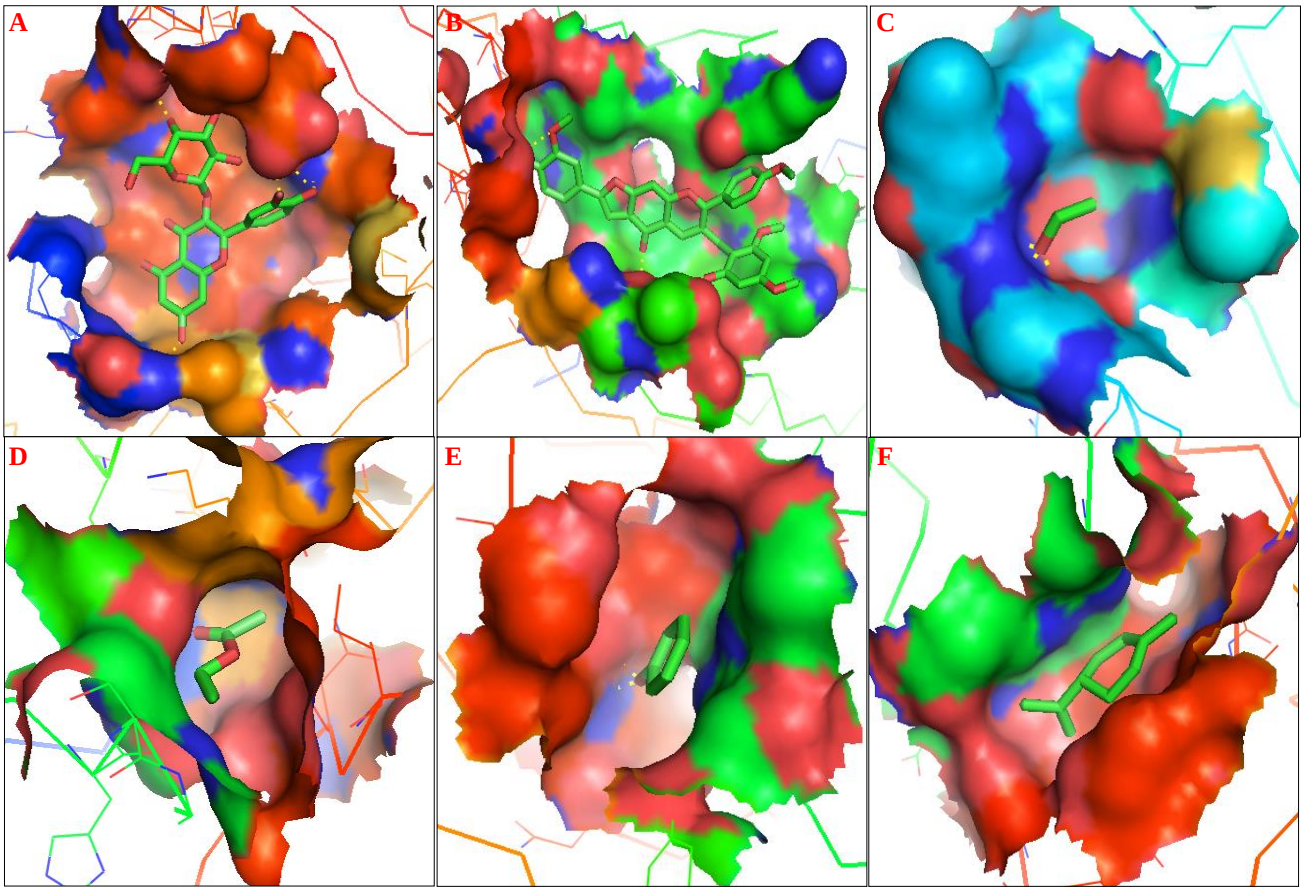

Fig. 2. Hydrophobic pocket of E-glycoprotein along with selected inhibitor molecules. Green structures represent small potential inhibitor molecules. A) hyperoside B) cissampeloflavone C) ethylene glycol D) eugenol E) phenol F) apigenin. The images of Figs. 2-3 were generated in Discovery Studio.

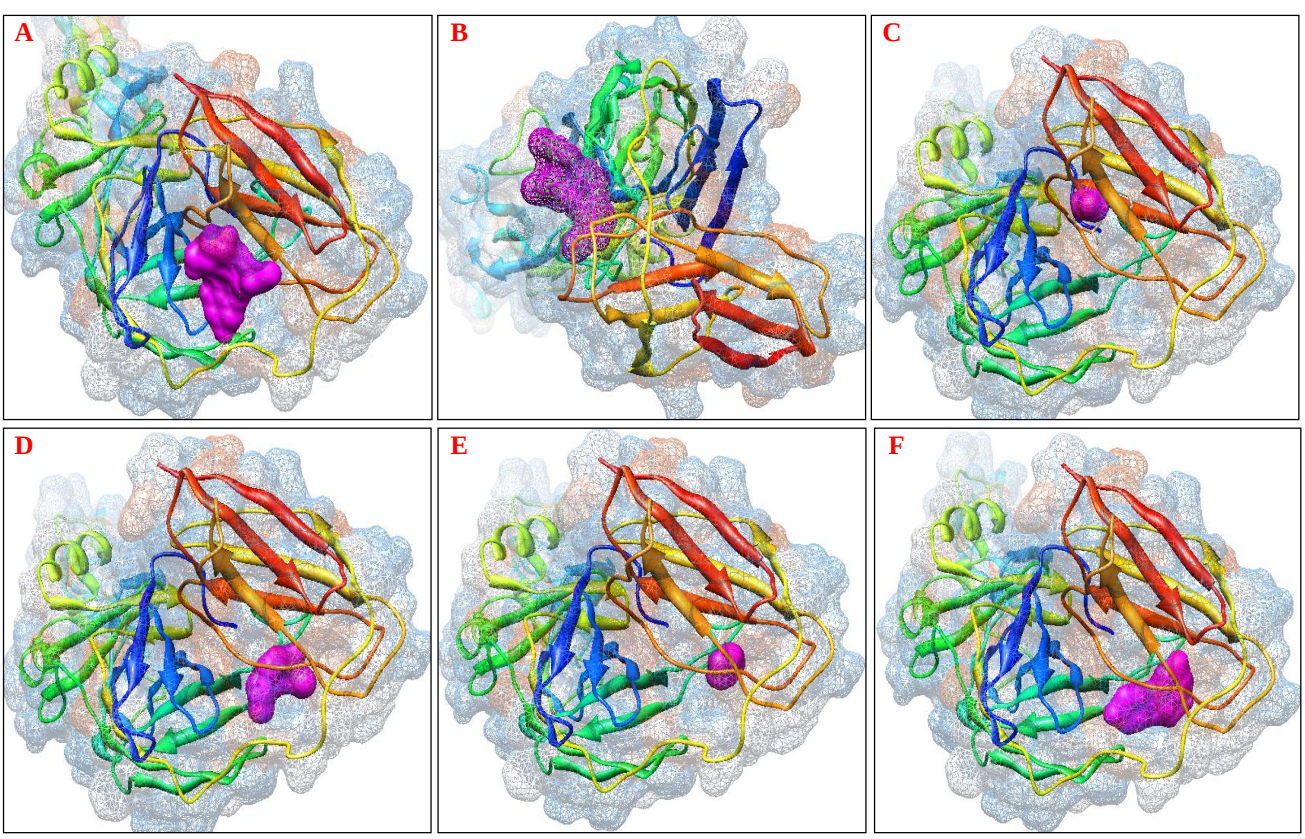

Fig. 3. Docked complexes of bioactive compounds with the binding pocket of E-glycoprotein; A) hyperoside B) cissampeloflavone C) ethylene glycol D) eugenol E) phenol F) apigenin.

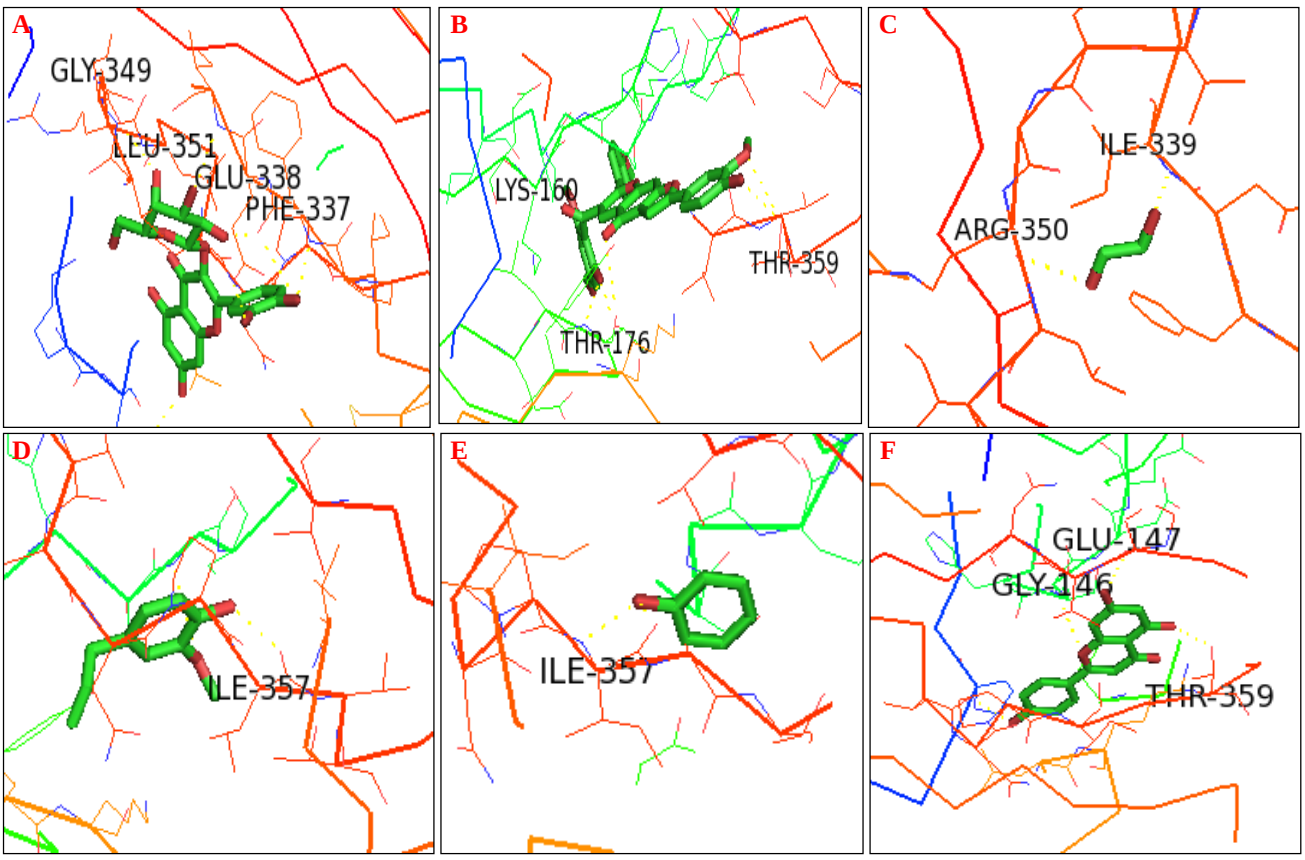

Fig. 4. Selected inhibitor molecules show hydrogen bonding with E-glycoprotein. A) hyperoside B) cissampeloflavone C) ethylene glycol D) eugenol E) phenol F) apigenin. The images of Figs. 4-5 were generated in Chimera 1.6.1 software.

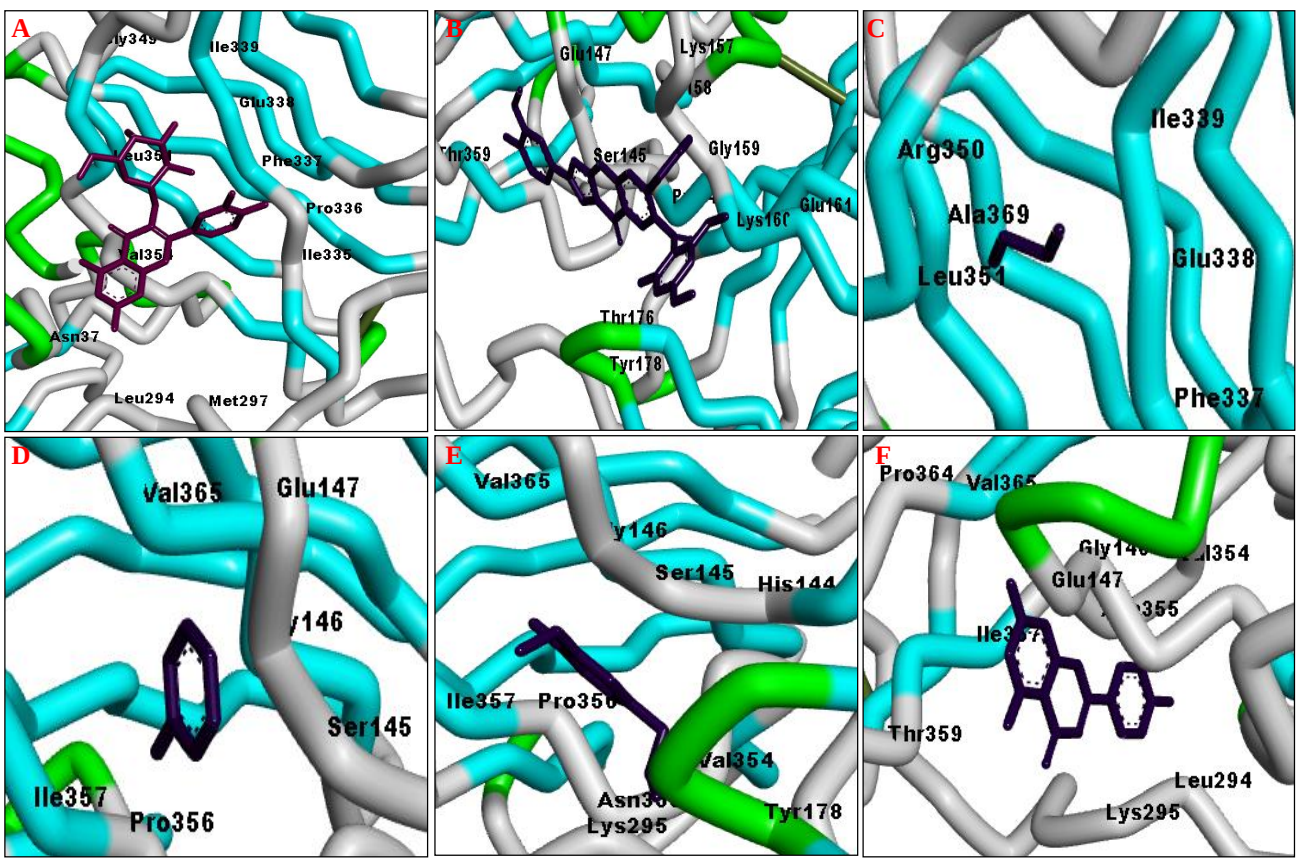

Fig. 5. The 3D crystal structure of E-glycoprotein shows residues close to the ligand molecule A) hyperoside B) cissampeloflavone C) ethylene glycol D) eugenol E) phenol F) apigenin.

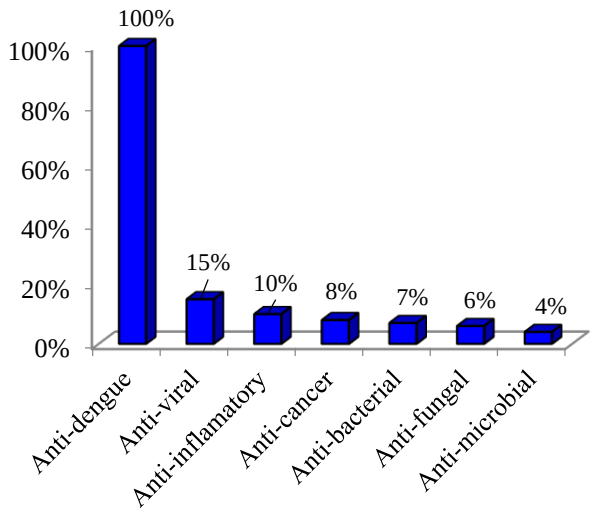

Fig. 6. Percentages of medicinal activities shown by selected plants.

From the six selected compounds, hyperoside is considered as a potential anti-dengue drug compound. The same method discussed earlier was utilized for docking of all the compounds with the E-glycoprotein active site. The selection of best conformation was based on the lowest binding energy of compounds in ten assessed conformations. Table 2 shows the QSAR descriptors of natural anti-dengue compounds and their energies. The conformation of each selected compound was analyzed after saving. For obtaining the interactions of the best conformation of the compound, PDB file of the protein was opened in the Discovery Studio. Hyperoside showed 5 hydrogen interactions and also have shown hydrophobic and ionic interactions respectively. However, cissampeloflavone showed 3 hydrogen interactions, whereas the ionic and hydrophobic interactions were also discovered. The ethylene glycol showed two hydrogen interactions as well as ionic and hydrophobic interactions. Single hydrogen interaction along with ionic and hydrophobic interactions were also shown by Eugenol. One hydrogen interaction was shown by phenol, where ionic and hydrophobic interactions were also displayed. It was found that 3 hydrogen interactions as well as ionic and hydrophobic interactions were investigated in Apigenin. Based on interactions with E-glycoprotein (target protein), maximum interactions with the target protein were shown by hyperoside. Although good interactions were shown by all the inhibitors but hyperoside showed the highest binding affinity. Five hydrogen interactions along with hydrophobic and ionic interactions were shown by hyperoside (lead compound) with the lowest binding pocket interactions' distance (3.5 Å). Hence, good interactions were shown by the hyperoside with our targeted protein presenting it a potential drug candidate against Dengue (Figs. 3-5).

Establishing quantitative structure activity relationship (QSAR) and drug scan: The data for six selected compounds was analyzed by QSAR to check the compounds having an exceptional symbol of attainment. The current QSAR study was conducted to identify and check the suitability of specified compound in the binding pattern of the selected target. Binding energy (kcal/mol), LogP and molar refractivity (MR), toxicity class, RMSD values of the currently selected compounds were calculated using hydration energy and analyzed with the ArgusLab4.0.1 program on Chem Draw. The server Binding Affinity Prediction of Protein Ligand (BAPPL) aids in the estimation of binding energy of protein ligand complexes presented in Table 2. In order to confirm the requirement of the compound as a better drug candidate, an online chemo informatics tool (admetSAR) was used to perform the drug scan. In the end, in order to assess the likeliness of the drug compound molecular profile, Lipinski's rule was utilized (Lipinski, 2004), which states that the molecular weight of phytochemical drugs must be <500, LogP (Compound's Lipophilicity) <5, hydrogen donor numbers <5, and hydrogen acceptor numbers <10. Molecular properties were shown by this rule which are important for the understanding the drug's pharmacokinetics in the body of a human, along with their metabolism, distribution, absorption and excretion. Investigation showed that all selected six phytochemical drug compounds passed the essential filters. Table 2 illustrates the compounds inquiries for drug suitability of six compounds such as molecular formula, exact mass, polar surface area, hydrogen accepter/donor, toxicity class, RMSD, boiling/melting points, Gibbs energy, LogP value and binding energies. After detailed analyses, a list of plants were compiled with the important anti-dengue compounds and their efficiency against dengue was checked using various bioinformatics approaches (Table 3). The list represents medicinal plants used in current study with their common names, family, medicinal uses, part used and phytochemical compounds with anti-dengue activities. The detailed investigations confirmed that most of the plants from various families have shown the availability of anti-dengue compounds like hyperoside, cissampeloflavone, ethylene glycol, eugenol, phenol and apigenin. The important plants with anti-dengue properties were *Andrographis paniculata, Azidarachta indica, Boerhaavia diffusia, Boesenbergia rotunda, Carica papaya, Chondrus crispus, Curcuma longa, Mimosa scabrella, Momordica charantia, Syzygium aromaticum* and *Quercus lusitanica.* Different plants parts are used for these herbal compounds such as leaves, stem, roots, fruits, seeds, bark or whole plant. The ethno-pharmacological uses of the listed plants have shown efficient activities such as antioxidant, anti-inflammatory, anticancer, anti-hepatitis, antibacterial and antiviral (anti-dengue) properties (Table 3; Fig. 6).

Discussion

Dengue infection recurs as a serious threat to life, with an annual increase in infection rates. As a result, there is a need to create effective compounds (anti-dengue) to combat this widespread illnesses (Nair *et al.*, 2010). A novel perspective model molecule emerges as a call for the pharmaceutical industry because drug candidates cannot be produced as a consequence of the lack of a natural model. For this reason, it is vital to obtain an understanding of plants, since innate sources are required for model molecule isolation (Drwal *et al.*, 2014). Previous investigations have suggested quercetin as a major inhibitor since they exhibit better ligand enzyme stability and interactions, and DENV-2 exhibits significant anti-viral activity in vero cells inversely (Senthilvel *et al.*, 2013).

Table 2. QSAR descriptors of natural anti-dengue compounds.

Chemical properties	Hyperoside	Cissampeloflavone	Ethylene glycol	Eugenol	Phenol	Apigenin
Molecular formula	$C_{21}H_{20}O_{11}$	$C_{34}H_{26}O_{10}$	$C_2H_6O_2$	C10H12O2	C_6H_60	$C_{15}h_{10}o_5$
Molecular weight (g/mol)	464.38	594.56	62.07	94.11	94.11	270.24
Exact Mass	464.095	594.153	46.0419	94.0419	94.0419	270.053
Polar surface area	210.51	137.8	40.46	20.23	20.23	90.9
Hydrogen Acceptor	8	10	2	1	1	5
Rotatable bond	4	8	1	0	0	1
Formal Charge	0	0	0	0	0	0
Hydrogen donor	0	0	0	0	0	0
Toxicity Class	5	4	5	3	3	5
RMSD	0.8	1	0.4	0.4	0.4	0.6
Boiling Point (K)	1599.43	1585.96	267.78	439.2	439.2	957.59
Melting Point (K)	1259.92	1229.33	134.03	282.5	282.5	785.56
Critical Temperature (K)	1333.41	1291.28	426.13	677.45	677.45	957
Critical Pres (Bar)	54.39	15.08	49.11	59.26	59.26	56.62
Critical Volume (cm^3/mol)	1067.5	1546.5	165.5	279.5	279.5	671.5
Gibbs Energy(KJ/mol)	-1163.79	-379.51	-139.04	-32.94	-32.94	-305.27
Log P	-1.39	2.72	0.09	1.64	1.64	1.9
MR (cm^3/mol)	109.79	166.05	12.8	27.09	27.09	73.51
Henrys Law	33.34-	24.2	1.45	4.64	4.64	17.78
Heat of formation(kJ/mol)	1772.48	-1015.77	-216.83	-96.48	-96.48	-559.68
Tpsa(Å)^2	206.6	129.98	9.23	20.23	20.23	86.99
x-score	(-133.01kcal/mol)	(-0.946kcal/mol)	(-12.266kcal/mol)	(-25.624kcal/mol	(-63.353kcal/mol)	(-20.887kcal/mol)
Binding energy(kcal/mol)	-2.23	-1.21	-1.23	-0.20	-0.27	-0.28
Elemental analysis	C:0.543 H:0.042 O:0.413	C:0.687 H:0.044 O:0.269	C:0.521 H:0.131 O:0.347	C:0.766 H:0.064 O:0.170	C:0.766 H:0.064 O:0.170	C:0.667 H:0.037 O:0.296
Residues interacting with Ligand (H Bonding)	Gly-349, Leu-351, Phe-337, Phe-337, Glu-338	Thr-176, Lys-160, Thr-359	Arg-350, Ile-339	Ile-357	Ile-357	Thr-359,Glu-157, Gly146
Lipinski's rule of five	Suitable	Suitable	Suitable	Suitable	Suitable	Suitable

Table 3. Medicinal plants and their phytochemical compounds with anti-dengue activities.

No.	Botanical name	Common names	Family names	Ethno-pharmacologicaluses	Parts used	Anti-Dengue compounds
1.	*Andrographis paniculata*	Creat, Kariyat, Indian Echinacea	Acanthaceae	It has a strong inhibitory effect against Dengue virus-2 and increases the platele level	Whole plant, leaves	Hyperoside
2.	*Alternanthera philoxeroides*	Alligatorweed	Amaranthaceae	It has shown a larvicidaleffects against*Aedes aegypti* mosquitoes, also used ir tuberculosis, measles and encephalitis B treatment	Leaves	Cissampeloflavone
3.	*Boesenbergia rotunda*	Ginger, Finger root	*Zingiberaceae*	It has antioxidant, anti-inflammatory, antibacterial, antiviral, anticancer properties and shown inhibitory activity on the replication of Dengue virus-2	Leaves	Ethylene glycol
4.	*Castanospermumaustral*	Black Bean	Fabaceae	This plant has antiviral activity and used in the treatment of dengue fever, HI and AIDS	Seeds	Eugenol
5.	*Chondrus crispus*	Irish moss, Red seaweeds	Gigartinaceae	The components found in this plant were more effective inhibitors of Dengue virus-2	Leaves	Phenol
6.	*Cissampelos pareira*	Velvetleaf	Menispermaceae	Methanolic extract of this plant have shown antiviral activities against dengue	Leaves	Apigenin
7.	*Gastrodia elata*	Gastrodia	Orchidaceae	The extract ofthis plant showed inhibitory activity on Dengue virus-2	Roots	Hyperoside
8.	*Euphorbia hirta*	Asthma weed	Euphorbiaceae	It has anti-bacterial, anti-viral and anti-fungal activities, treat bronchial asthma, laryngeal spasm, syphilis, and inhibits dengue	Leaves	Cissampeloflavone
9.	*Uncaria tomentosa*	Cat's claw	Rubiaceae	The hydro-alcoholic extract has anti-viral, anti-inflammatory, anti-microbial and anti-bacterial activities, used in the treatment of DANV-2, also used to treat wounds, treat stomach problems and cancer	Vine bark, root	Hyperoside
10.	*Kaempferia parviflora*	Black ginger	Zingiberaceae	It has anti-viral, anti-inflammatory and anti-oxidant activities	Leaves and stem	Hyperoside
11.	*Rhizophora apiculata*	Mangrove	Rhizophoraceae	Petroleum ether extract has larvicidal activity against *A. aegypti* mosquito, shown inhibitory activities against flatulence; epilepsy, small pox, asthma, diabetes, rheumatism, stomach aches, fever, malaria, cholera and hepatitis	Leaves and stem	Cissampeloflavone
12.	*Mimosa scabrella*	Mimosa, Bracatinga	Fabaceae	The Galactomanians extracted from *Mimosa scabrella* seeds showed activities against Dengue virus-1 *In vitro* and *In vivo*	Seeds	Cissampeloflavone
13.	*Leucaena leucocephala*	White lead tree	Fabaceae	This plant has antiviral activity against yellow fever and dengue	Seeds	Eugenol
14.	*Phyllanthus urinaria*	Chamber bitter, Gripe weed	Phyllanthaceae	It has shown anti-cancer activities by inducing apoptosis. Its methanolic extract show anti-dengue activities	Leaves	Ethylene glycol
15.	*Phyllanthuswastonii*	-	Phyllanthaceae	The methanolic extract of this plant has anti-dengue activity	Leaves	Ethylene glycol
16.	*Cladosiphon okamuranus*	Brown seaweed	Chordariaceae	Fucoidan extract showed strong anti-dengue, antitumor activities and is a potential therapeutic agent for patients with Adult T-Cell leukemia (ATL)	Leaves	Cissampeloflavone
17.	*Meristiella gelidium*	Tengusa, Makusa	Solieriaceae	The extract and the fraction were more effective inhibitors of Dengue Virus-2	Leaves	Apigenin
18.	*Oldenlandia affinis*	Small-egg plant	Rubiaceae	Showed inhibitory activities against dengue viral NS2B and NS3 protease	Leaves, stems and roots	Phenol
19.	*Quercus infectori*	Magic Nut, Masikai	Fagaceae	Methanol extracts of*Quercus infectori* have strongest inhibitory activity agains Dengue Virus-2	Leaves	Apigenin
20.	*Piper longum*	Papal	Piperaceae	The plant has shown control activities against *A. aegypti*	Fruits	Hyperoside

Table 3. (Cont'd.).

No.	Botanical name	Common names	Family names	Ethno-pharmacological uses	Parts used	Anti-Dengue compounds
21.	*Pimpinella anisum*	Anisuan, Aniseed	Apiaceae	Showed insecticidal action against *A. aegypti* larvae	Whole plant	Phenol
22.	*Curcuma longa*	Turmeric	Zingiberaceae	Ethyl acetate extract of *Curcuma longa* plant possess activity in inhibiting dengue fever	Stem	Eugenol
23.	*Murraya koenigii*	Curry leaf	Rutaceae	Curry leaves possesses anti-diabetic, antioxidant, hepatoprotectiveproperties, strengthen the bones, improve digestion, and strengthen hair roots	Leaves	Hyperoside
24.	*Caesalpinia pulcherrima*	Peacock flower, Red bird o paradise	Fabaceae	Leaves are used as repellent for *A. aegypti*	Leaves	Eugenol
25.	*Momordica charantia*	Bitter melon	Cucurbitaceae	Methanolic extract of *M. charantia* plant have inhibitory effect on Dengue Virus-1	Leaves, stem, fruit, and resin	Hyperoside
26.	*Murraya koenigii*	Kari patah or Kariapat	Rutaceae	The acetone and petroleum ether extracts of leaves has larvicidal effect again *A. aegypti*	Leaves	Cissampeloflavone
27.	*Piper sarmentosum*	Papal or Pippli	Piperaceae	The ethanolic extract of plant have shown larvicidal activities against *A. aegypti* mosquitoes	Fruit, root and stem	Hyperoside
28.	*Houttuynia cordata*	Fish mint, Lizard tail	Saururaceae	Ethanol extracts from *Houttuynia cordata* plant shows an anti-dengue effect with 35.99% inhibition against Dengue Virus-2	Leaves	Hyperoside
29.	*Carica papaya*	Papita	Caricaceae	Aqueous extract of leaves of *Carica papaya* plant exhibited potential activity against Dengue fever	Leaves	Hyperoside
30.	*Hippophae rhamnoides*	Sea-buckthorn	Elaeagnaceae	The extracts ofleaves showed anti-dengue activity against DENV-2	Leaves	Cissampeloflavone
31.	*Piper ribesoides*	Papal or Pippli	Piperaceae	The ethanolic extract of *Piper ribesoides* plant showed larvicidal activity against *A. aegypti* mosquitoes	Fruit, root and stem	Ethylene glycol
32.	*Pemphis acidula*	Mentigi	Lythraceae	Crude leaf extracts has larvicidal, ovicidal and repellent properties against *A. aegypti*	Leaves	Eugenol
33.	*Azidarachta indica*	Neem	Meliaceae	The aqueous extract of neem leaves has inhibitory effect on the replication of Dengue virus-2	Leaves	Phenol
34.	*Boerhaavia diffusia*	Spreading Hogweed,	Nyctaginaceae	The plant showed antiviral efficacy against phyto-pathogenic viruses	Roots, leaves and seeds	Apigenin
35.	*Tinospora cordifolia*	Amrita, Gilu, Giluncha	Menispermaceae	The plant extract is used for diabetes, allergic rhinitis, high cholesterol, cance rheumatoid arthritis, hepatitis, dengue fever, syphilis and gonorrhea	Stem and leaves	Ethylene glycol
36.	*Andropogon citratum*	Citronella grass	Poaceae	Showed effectiveness in repelling *A. aegypti*	Seeds	Eugenol
37.	*Psidium guajava*	Guava leaves	Myrtaceae	The leaf extracts reduce the growth of DENV	Leaves	Phenol
38.	*Ervatamia coronaria*	Pinwheel Jasmine	Apocynaceae	*Ervatamia coronaria* plant is utilized as a herbal remedy to protect the mosqui bite	Leaves	Apigenin
39.	*Syzygium aromaticum*	Laung, clove	Myrtaceae	*Syzygium aromaticum* vital oil is used as insect repellents including *Ag aegypti*	Seeds	apigenin
40.	*Quercus lusitanica*	Mazu Phal	Fagaceae	The methanolic extract of *Quercus lusitanica* have shown inhibitory effect on the replication of DANV-2	Leaves	Eugenol

DENV-2 has a large binding cavity, so there are a number of active sites available for docking. Six phytochemical ligands from our docking examination indicated magnificent docking interactions with energies to DENV-2 (Table 2). These phytochemical agents were used to target the active site of the DENV-2 E protein. Phytochemicals small molecules can inhibit viral proteins by binding to glycoprotein active site of the protein. The results of the molecular docking provided a 2-D bioactive complex structure that specifically interacted with conserved target site residues. Interestingly, it was observed that Catechin, Laurifolin, Cianidanol had the highest binding energy to NS4B in DENV-1,2,4; which is due to the formation of more hydrogen bonds with amino acid residues at the binding site of the receptor. On the bases of results it was observed that the compound, particularly Catechin had anti-dengue activity (Paul *et al*., 2016). The non-competitive inhibitor doxycycline showed a lower binding energy -5.15 kcal/mol than the predicted competitive inhibitors meclofenamic acid and comparable inhibitory inhibitors -3.64 and -3.21 kcal/mol respectively, against the NS2B-NS3 protease from DENV-2. Structural analyses showed compound doxycycline interacting with Lys74; an important amino acid residue linked to a specific allosteric region and linked to one of the Asp75 catalytic triads. Compounds meclofenamic acid and rolitetracycline showed direct binding with two of the His51 and Ser135 catalytic trials predicted as competitive inhibitors (Othman *et al*., 2017).

It has been determined that the two ligands are the most suitable lead compounds, 1) 7-methyl-guanosine-5'-triphosphate-5 '- (2'-O-methyl)-Guanosine and 2) Boctyglucucide had binding energy of (-123.2 and -84.61) respectively, for NS5 protein and envelope protein (Raikar *et al*., 2017). ADMET properties and molecular docking results revealed 9 phytochemicals with potential inhibitory properties against Dengue virus namely, Isosilybin, Silydianin, Derrisin, Silymarin, Mundulinol, Narlumicine, Flavobion, Oxysanguinarine and Isopomorphine and to assess their *In vitro* and *In vivo* work can be considered as additional. These phytochemicals exhibited binding affinity ≥ –8 kcal/mol against Dengue virus 4-NS4B. The highest binding energy was shown for Palmatine -103.076 kcal/mol, delphinidin chloride -109.187 kcal/mol, squalene -109.975 kcal/mol and marmin -91.84 kcal/mol, 98.74 kcal/mol, while d-limonene and allicin exhibit a minimum binding energy to the binding sites of Dengue protein (PDB id. 3L6P) (Badoni *et al*., 2015).

The present research was conducted to retrieve new drug molecules that can work as anti-dengue inhibitors by utilizing structure-based drug design methodology. E-glycoprotein was selected as a target in order to find new drugs. Hydrophobic cavity of DENV-2 E protein (Figs. 3-5A-F) showed a potential target for small-molecule inhibitors (Powers & Setzer, 2016). Six phytochemical drug compounds were chosen as a ligand, which were found effective against dengue virus. Every ligand was analyzed along with the target molecule complexes that were formed. Their energy values and binding affinities to the target were also assessed. On the bases of outcomes of current study, a lead compound was recognized. Six selected compounds were docked to find the best inhibitor against Dengue. All inhibitors interacted well with the target E-glycoprotein, but phytochemical compound hyperoside showed the best docking complex with the target receptor. For this reason, the best of all other compounds is hyperoside and could be recommended as a drug for investigations in future. Anti-dengue inhibitors versus QSAR descriptors have been established to find addictive tendencies. Based on various descriptors, dengue virus showed dependence on drug activity. Diverse electronic and steric descriptors have been utilized to assess the association activity of the drug to the structure. The current study involved identification of six phytochemicals compounds with excellent docking properties with E-glycoprotein active site of DENV envelope protein. The results of this study reflect prior docking studies that showed phytochemicals to be astounding docking ligands to dengue protein targets (Powers *et al*., 2016). This type of method aids to design the new drug with higher efficiency and inhibitors to Dengue virus.

Conclusion

The current study achieved the desired goal by using a structure-based drug designing that provides the best inhibitor to serve as a powerful agent against DANV. The prominence of this study is reflected in the best definition of the six compounds as balanced DANV inhibitors. The lead compound hyperoside has been described in order to improve the effectiveness of antidepressant drugs due to the virus resistance. Proposed study regarding the potential drug designing against DENV might give important and effective data for the continuous exploration, innovation and design of new potential anti-viral drugs against DANV. The current study proposed the further formulations of these compounds and clinical trials to check their efficacy *In vivo*.

References

Agnaniet, H., E.J. Mbot, O. Keita, J.A. Fehrentz, A. Ankli, A. Gallud, M. Garcia, M. Gary-Bobo, J. Lebibi, T. Cresteil and C. Menut. 2016. Antidiabetic potential of two medicinal plants used in Gabonese folk medicine. *BMC Complement. Altern. Med*., 16: 71. doi: 10.1186/s12906-016-1052-x.

Anonymous. 2016. World Health Organization-WHO. Available from: http://www.who.int/topics/dengue/en/.

Babar, M., S.Z. Najam-us-Sahar, M. Ashraf and A.G. Kazi. 2013. Antiviral drug therapy- exploiting medicinal plants. *J. Antivir. Antiretrovir*., 5: 028-036.

Badoni, H., S. Painuli and P. Semwal. 2015. *In silico* screening of phytoactive components against Junin, Hanta, Dengue, Marburg and Ebola Viruses. *J. Chem. Pharm. Res*., 7(3): 209-224.

Bhatt, S., P.W. Gething and O.J. Brady. 2013. The global distribution and burden of dengue. *Nature*, 496: 504.

Cheng, F., W. Li, Y. Zhou, J. Shen and Z. Wu. 2012. admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *J. Chem. Inf. Model.,* 52: 3099-3105.

Drwal, M. N., P. Banerjee, M. Dunke, M. R. Wettig and R. Preissner. 2014. ProTox: a web server for the in silico prediction of rodent oral toxicity. *J. Nucl. Acids. Res*., 2: 10-20.

Hao, D.C., P.G. Xiao, H.Y. Ma, Y. Peng and C.N. He. 2015. Mining chemodiversity from biodiversity: pharmacophylogeny of medicinal plants of Ranunculaceae. *Chin. J. Nat. Med.,* 13(7): 507-520.

Irwin, J.J., T. Sterling, M.M. Mysinger, E.S. Bolstad and R.G. Coleman. 2012. ZINC: A free tool to discover chemistry for biology. *J. Chem. Inf. Model*., 52: 1757-1768.

Kiat, T.S., R. Pippen, R. Yusof, H. Ibrahim, N. Khalid and N.A. Rahman. 2006. Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, *Boesenbergia rotunda* (L.), towards dengue-2 virus NS3 protease. *Bioorg. Med. Chem. Lett*., 16(12): 3337-3340.

Lee, S.H., Y.Q. Tang, A. Rathkrishnan, S.M. Wang, K.C. Ong, R. Manikam, B.J. Payne, I.B. Jaganath and S.D. Sekaran. 2013. Effects of cocktail of four local Malaysian medicinal plants (*Phyllanthus* spp.) against dengue virus 2. *BMC Complement. Altern. Med*., 13: 192. doi: 10.1186/1472-6882-13-192.

Lipinski, C.A. 2004. Lead and drug like compounds: the rule-of-five revolution. *Drug. Discov. Today J. Technol*., 1(4): 337-341.

Mizumoto, K., K. Ejima, T. Yamamoto and H. Nishiura 2014. On the risk of severe dengue during secondary infection: a systematic review coupled with mathematical modeling. *J. Vector Borne Dis.,* 51: 153.

Morries, G.M., R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell and A.J. Olson. 2009. AutoDoch4 and AutoDocktool4: automated docking with selective receptor flexibility. *J. Comp. Chem*., 16: 2785-2791.

Okonkwo, E.E. 2012. Traditional Healing Systems among Nsukka Igbo. *J. Tourism Heritage Stud*., 1: 69-81.

Othman, R., R. Othman, A. Baharuddin, N.R. Ramakrishnan, N.A. Rahman, R. Yusof and S.A. Karsani. 2017. Molecular docking studies of selected medicinal drugs as dengue virus-2 protease inhibitors. *Sains Malaysiana*. 46(10): 1865-1875.

Paul, A., A. Vibhuti and S. Raj. 2016. Molecular docking NS4B of DENV 1-4 with known bioactive phyto-chemicals. *Bioinformation.* 12(3): 140-148.

Powers, N.C. and N.W. Setzer. 2016. An in-silico investigation of phytochemicals as antiviral agents against dengue fever. *Comb. Chem. High Throughput Screen.,* 19(7): 516-536.

Qaddir, I., N. Rasool, W. Hussain and S. Mahmood. 2017. Computer-aided analysis of phytochemicals as potential dengue virus inhibitors based on molecular docking, ADMET and DFT studies. *J. Vector Borne Dis*., 54: 255-262.

Qamar, T., A. Mumtaz, U.A. Ashfaq, S. Azhar, T. Fatima, M. Hassan, S.S. Hussain, W. Akram and S. Idrees. 2014. Computer aided screening of phytochemicals from Garcinia against the Dengue NS2B/NS3 Protease. *Bioinformation*. 10: 115-118.

Raikar, S., N. Patil, M. Kulkarni, N. Kulkarni, Deepak. A. Yaraguppi, Uday. M. Muddapur and Zabin. K. 2017. Bagewadi. In silico Study on Inhibition of NS5 Protein of Dengue Virus. Research & Reviews: *A.J. Bioinform*. *RRJoBI.,* 4(2): 28-43.

Rothan, H.A., M. Zulqarnain, Y.A. Ammar, E.C. Tan, N.A. Rahman and R. Yusof. 2014. Screening of antiviral activities in medicinal plants extracts against dengue virus using dengue NS2B-NS3 protease assay. *Trop. Biomed*., 31(2): 286-96.

Schneidman-Duhovny, D., Y. Inbar, R. Nussinov and H.J. Wolfson. 2005. PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucl. Acids Res.*, 33: 363-367.

Senthilvel, P., P. Lavanya, K.M. Kumar, R. Swetha, P. Anitha, S. Bag, S. Sarveswari, V. Vijayakumar, S. Ramaiah and A. Anbarasu. 2013. Flavonoid from *Carica papaya* inhibits NS2B-NS3 protease and prevents Dengue 2 viral assembly. Biomedical Informatics. *Bioinformation*, 9(18): 889-895.

Wang, M., L. Tao and H. Xu. 2016. Chinese herbal medicines as a source of molecules with anti-enterovirus 71 activities. *Chin. Med*., 11: 2. doi: 10.1186/s13020-016-0074-0.

(Received for publication 22 March 2019)