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Computational screening of *Carica papaya* natural bioactive compounds against the dengue NS2B/NS3 protease

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Abstract

Dengue virus infection is a serious public health concern all over the world including Pakistan. Currently, there is no effective treatment to control dengue infection. So, there is a dire need to identify safe and low cost drug against dengue virus. This study was planned to computationally screen the phytochemicals of *C. papaya* against dengue NS2B/NS3 serine protease which is essential and specific for the virus replication. For this purpose, a library of 900 bioactive compounds were screened against NS2B/NS3 to find out potential candidates against dengue NS2B/NS3. Nine compounds (1-Hydroxy-2-propanone, 2-methyl-propanoic acid, baicalein, 2-methyl-butanoic acid, epigallocatchin, fisetin, genistein, catechin and protocatechuric acid) out of 900 were ranked on the basis of S-score (-5.7626, -6.8558, -7.2808, -6.4986, -7.5166, -13.1129, -7.5972, -9.0272, -7.3422 and rmsd value 0-9580, 1.5403, 2.1917, 1.9903, 2.9203, 3.4554, 2.4394, 3.1414 and 1.8594) and high bonding interaction with NS2B/NS3 serine protease. The screened compounds were further filtered through ADMET profiling and Lipinski's rule of five. Three compounds: Epigallocatchin, catechin and protocatechuric acid having interaction with active residues His51, Asp75, Ser135 were predicted. The analysis shows potential of phytochemicals from *C. papaya* for possible anti-dengue agent in pharmaceutical as well as nutraceutical industry.

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Introduction

Dengue virus (DENV) is leading health problem worldwide and there is no effective treatment to overcome this problem. According to the latest study more than 50-100 million infection cases were reported per year (Goodwin *et al.,* 2005; Erbel *et al.*, 2006). Several countries especially Africa, South America, Central America and the tropical region of Asian countries like India and Pakistan are at high risk of dengue virus (Qamar *et al.*, 2016). Dengue fever is caused by the *Flavivirus* and have five serotypes (DENV 1, 2, 3, 4 and 5).

The *Flavivirus* are mostly colonized in sultry and moderate regions of the biosphere and cause several contagions in human diseases (Sousa *et al.,* 2015).

Dengue infections in monkey, mice and human, spread through the bite of two carrier mosquitoes *Aedes albopictus* and *Aedes aegypti*. The genomic size of dengue virus is 10-11 kb consisting of 10 proteins (Yang *et al.,* 2010; Wadood *et al.,* 2014). Three structural proteins (capsid/core protein, E protein and membrane associated protein) and seven nonstructural proteins (NS1-NS5).

Dengue NS3 and NS5 protein play an energetic role in viral replication (Qamar *et al.*, 2016). Dengue NS2B and NS3 protease interact with each other and form a pro-complex NS2B/NS3, which have a role to cleave viral proteins to perform their function (Warrilow *et al.,* 2012). So slightly disruption in pro-complex NS2B/NS3 is reported to inhibit viral replication. NS2B/NS3 complex is to be an important target for the assessment and screening of anti-dengue and antiviral drugs candidates (Idrees and Ashfaq, 2012).

Medicinal plants contain large number of natural occurring bioactive compounds such as saponins, polyphenolics, terpenoids, flavonoids, peptides, thiophenes, coumanrins, ploynes, alkaloids, organosulfur compounds, limonoids and many other bioactive compounds (Debnath *et al.*, 2003; Bibi *et al.*, 2008). These bioactive compounds have low side effects and more effective inhibiters against NS2B/NS3 of dengue virus RNA (Rothan *et al.*, 2014; Takshak *et al.*, 2018). This study was planned to screen bioactive compounds of *C. papaya* to find out their potential inhibitors of dengue infection.

The *C. papaya* plant has immunity enchantment bioactive compounds which are used against viral spreading (Mukhtar *et al.,* 2008). *C. papaya* plant belongs to the family *C.eae* in all parts of the world.

It is medicinal plant, all parts of the plant leaves, flowers, roots and stem have lot bioactive compounds (Wall *et al.*, 2006; Otsuki *et al.*, 2010). More than 900 bioactive compounds of different classes (Aroma compounds, Xanthones, Terpenoids, Tannins, Steroids, Saponins, lignin, carbohydrates, Aromatic) were present in *C. papaya* (Asghar *et al.*, 2016).

This project was designed for computational screening of *C. papaya* phytochemicals against dengue NS2B/NS3 to treat dengue virus infection.

Materials and methods

Structure retrieval and optimization

The 3D structure of dengue virus NS2B/NS3 protein was retrieved from protein data bank (PDB) using PDB ID: 2FOM. The retrieved structure of dengue virus NS2B/NS3 protein was optimized by removing ligand, 3D protonation, solvent residues and energy minimization through molecular operating environment (MOE).

Preparation of C. papaya ligand library

About 900 bioactive compounds of *C. papaya* were retrieved from MAPS (Ashfaq *et al.*, 2013), MPD3 (Mumtaz *et al.*, 2017; Taj *et al.*, 2019) and Pub Chem database (Bolton *et al.*, 2008; Velmuruga *et al.*, 2014). To scan the inhibitors, molecular docking have been performed against dengue NS2B/NS3 via MOE (Roy and Luck, 2007).

NS2B/NS3 dengue protein residues determination

The residues like His51, Asp75, Ser135 that participate in the interaction of NS2B/NS3 have been recognized and were selected using site finder tool of MOE (Frimayanti *et al.*, 2011; Idrees and Ashfaq, 2014).

Molecular docking

Ready-to-dock library of phytochemicals was docked with interacting residues of NS2B/NS3 through MOE. Following were the parameters set for docking: placement: triangle matcher; re-scoring: London DG; retain: 10; refinement: Forcefield; re-scoring 2: London DG and retain 10 (Taj *et al.*, 2019).

The structure of bioactive compounds were nominated on the basis of lower values of RMSD and higher S-score. Phytochemicals having higher values of S-score and lower value of (RMSD) were selected for ADMET analysis (Ma *et al.*, 2013).

ADMET profiling and in silico analysis of drug likeness properties

The nine best docking score bioactive compounds were further analyzed on the basis of Lipinski's rule of five (Ro5) (Lipinski *et al.*, 2004). In order to evaluate drug like characteristics the candidates were subjected to Swiss ADME software (Daina *et al.*, 2017). Calculation of ADMET properties i.e. toxicity, absorption and metabolism are significant sign of drug candidate's fate, behavior and level of toxicity in human body.

Results

Molecular docking and database screening study

The library of bioactive compound was docked against the NS2B/NS3 serine protease and docked compounds were ranked on the basis of S score and high bonding interaction with NS2B/NS3. Out of 900 docked compounds, nine top ranking compounds (1-Hydroxy-2-propanone, 2-methyl-propanoic acid, baicalein, 2-methyl-butanoic acid, epigallocatchin, fisetin, genistein, catechin and protocatechuric acid) were selected. These nine compounds have docking score -5.7626, -6.8558, -7.2808, -6.4986, -7.5166, -13.1129, -7.5972, -9.0272, -7.3422 and rmsd value 0-9580, 1.5403, 2.1917, 1.9903, 2.9203, 3.4554, 2.4394, 3.1414 and 1.8594 respectively. Most of bioactive compounds i.e. three out of top nine, tend to exhibit strong interaction with His51, Asp75, Ser135 (Fig. 1).

ADMET analysis

The selected top nine *C. papaya* compounds followed the Ro5 (Table 2). All the candidate bioactive compounds were subjected to ADME to assess them for their drug like properties (Table 3).

Table 1. Top nine bioactive compounds interaction detail with NS2B/NS3 dengue virus protein.

SN	Compound name	Docking Score	RMSD value	Residues
A	1-Hydroxy-2-propanone	-5.7626	0-9580	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
B	2-methyl-propanoic acid	-6.8558	1.5403	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
C	Baicalein	-7.2808	2.1917	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
D	2-Methyl-butanoic acid	-6.4986	1.9903	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
E	Fisetin	-7.5166	2.9203	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
F	Epigallocatchin	-13.1129	3.4554	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
G	Genistein	-7.5972	2.4394	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
H	Catechin	-9.0272	3.1414	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
I	Protocatechuric acid	-7.3422	1.8594	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151

Table 2. Lipinski's rule for *C. papaya* bioactive compounds.

SN	Compound name	MLogP	Number of HBA	Number of HBD	Molecular weight (g/mol)
Lipinski's rule of five		<5	<10	<5	<500
A	1-Hydroxy-2-propanone	0.569	1	0	88.106
B	2-methyl-propanoic acid	0.727	2	1	88.106
C	Baicalein	2.240	4	5	270.240
D	2-Methyl-butanoic acid	1.117	2	1	103.133
E	Fisetin	2.305	5	4	286.239
F	Epigallocatchin	2.329	10	5	458.375
G	Genistein	2.162	5	5	272.256
H	Catechin	1.642	6	5	290.271
I	Protocatechuric acid	0.796	4	2	154.121

Table 3. ADMET profiling results of top nine *C. papaya* bioactive compounds.

Compound name	Blood-brain barrier	$CaCO_2$ permeability	P-glycoprotein inhibitor	Renal organic cation transporter	Human intestinal absorption
Absorption					
A	BBB+	$CaCO_2$-	NI	T	HIA+
B	BBB+	$CaCO_2$-	NI	T	HIA+
C	BBB+	$CaCO_2$-	NI	T	HIA+
D	BBB+	$CaCO_2$-	NI	T	HIA+
E	BBB+	$CaCO_2$-	NI	T	HIA+
F	BBB+	$CaCO_2$-	NI	T	HIA+
G	BBB+	$CaCO_2$-	NI	T	HIA+
H	BBB+	$CaCO_2$-	NI	T	HIA+
I	BBB+	$CaCO_2$-	NI	T	HIA+
Compound name	CYP450 2C9 inhibitor	CYP450 IA2 inhibitor	CYP450 2C19 inhibitor	CYP450 2D6 inhibitor	CYP450 3A4 inhibitor
Metabolism					
A	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor
B	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor
C	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor
D	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor
E	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor
F	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor
G	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor
H	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor
I	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor	Non inhibitor

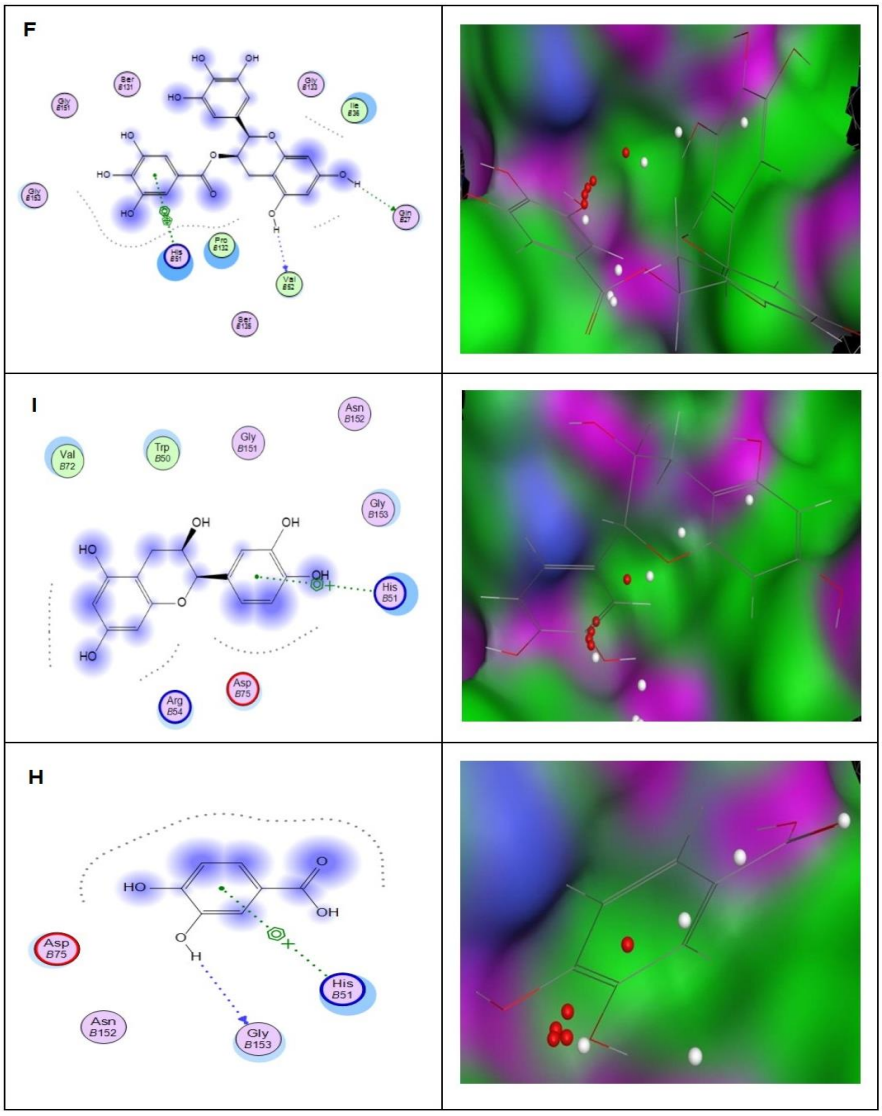

Fig. 1. 2D and 3D interactions of bioactive *C. papaya* compounds (ligands) with dengue NS2B/NS3 serine protease.

Discussion

Protein binding capacity of a compound is an important approach to find its potential to be used as pharmacological agent. Different approaches like structure based virtual screening, screening nature compounds, virtual screening and scaffold hopping, ligand based virtual screening, non-competitive binding, pepdidomimetics and small compounds libraries had been used to find out the inhibitors for dengue virus NS2B/NS3 protein. In the past several peptides, synthetic small molecules, cyclopeptides and natural inhibitors of DV NS2/NS3 have been stated but all these inhibitors may have high toxicity and weak bonding with the DV NS2/NS3 protease. Idrees *et al* designed different peptides, synthetic small molecules, cyclopeptides and natural inhibitors and docked against DV NS2B/NS3 protease. Only seven of them interacted with DV NS2B/NS3. Their results need *in vitro* investigation and optimization to confirm their efficacy (Wei *et al.*, 2007; Powell *et al.*, 2013). The current study highlights importance of *C. papaya* phytochemicals against dengue NS2B/NS3. About 900 bioactive compounds of *C. papaya* were docked with the active site of dengue NS2B/NS3 and nine compounds like 1-Hydroxy-2-propanone, 2-methyl-propanoic acid, baicalein, 2-methyl-butanoic acid, epigallocatchin, fisetin, genistein, catechin and protocatechuric acid had potential interaction with His51, Asp75, and Ser135. The compounds were ranked on the basis of S-Score, RMSD value and bonding interaction with active residues (Araujo *et al.*, 2012). Nine compounds A, B, C, D, E, F, G, H and I shows significant interaction with active residues of dengue NS2B/NS3.

Map view interaction gives a better understanding of the interaction of *C. papaya* bioactive compounds with His51, Asp75, Ser135 of dengue NS2B/NS3 serine protease. Compounds (1-Hydroxy-2-propanone, 2-methyl-propanoic acid, baicalein, 2-methyl-butanoic acid, epigallocatchin, fisetin, genistein, catechin and protocatechuric acid) have docking score -5.7626, -6.8558, -7.2808, -6.4986, -7.5166, -13.1129, -7.5972, -9.0272, -7.3422 and RMSD value 0.9580, 1.5403, 2.1917, 1.9903, 2.9203, 3.4554, 2.4394, 3.1414 and 1.8594 respectively.

The compound F has highest docking score, hydrogen bond donor range, HB (A), MW, $CaCO_2$, P-glycoprotein inhibitor, permeability, renal organic cation transporter, CYP450 2C9 inhibitor, carcinogens, AMES toxicity, CYP450 2D6 inhibitor, blood brain barrier, human intestinal absorption, CYP450 3A4 inhibitor and CYP450 2C19 inhibitor. Similarly, compounds H and I showed greater bonding interaction with Asp75, Ser135 residue, high docking score, Logp, RMSD value, residues that bind with ligands, HB (D), HB (A), MW, , $CaCO_2$ permeability, renal organic cation transporter, CYP450 2C9 inhibitor, carcinogens, AMES toxicity, CYP450 2D6 inhibitor, blood brain barrier, human intestinal absorption, CYP450 3A4 inhibitor, P-glycoprotein inhibitor and CYP450 2C19 inhibitor. Therefore, these three compounds of *C. papaya* like epigallocatchin, catechin and protocatechuric were selected for NS2B/NS3 serine protease inhibition. So these compounds are more effective to inhibit the dengue virus NS3 protease activity that ultimately reduces the dengue virus infection.

Conclusion

Dengue fever is leading health problem worldwide and there is no effective treatment to overcome this problem. In the present study focuses on *C. papaya* bioactive compounds against dengue virus NS2B/NS3 serine protease. Nine compounds 1-Hydroxy-2-propanone, 2-methyl-propanoic acid, baicalein, 2-methyl-butanoic acid, epigallocatchin, fisetin, genistein, catechin and protocatechuric acid out of 900 can be utilized as strong drug and potential candidates against NS2B/NS3 of dengue virus. Three bioactive compounds of *C. papaya* like epigallocatchin, catechin and protocatechuric were found to be potential inhibitors of NSB/NS3. These compounds will be helpful in manufacturing pharmacological agents in future to reduce and control dengue virus infection.

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