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Molecular docking and pharmacokinetics study for selected leaf phytochemicals from *Carica papaya* Linn. against dengue virus protein, NS2B/NS3 protease

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ABSTRACT

The Culex mosquito-transmitted dengue virus (DENV) under genus Flavivirus, a member of Flaviviridae family, cause dengue fever worldwide also in many parts of India. At present, antidengue drugs or therapies from natural products of the leaf extracts of papaya plant, *Carica papaya* Linn. is interesting research. The present computational prediction was attempted to detect inhibitory potential of established ten common phytoconstituents of leaf of *C. papaya* against the protein of dengue virus (NS2B/NS3 protease) through molecular docking and pharmacokinetic study. The NS2B/NS3 protease (receptor) was obtained (PDB: 2FOM) from European Protein databank. The phytochemicals ten numbers were used as ligands in this predictive study. The information of these phytochemicals was obtained from PubChem database. The software viz. PyRx (Version 0.8) and ADMET-SAR online tool were used for the study of molecular docking as well as pharmacokinetics study. The present results indicate that natural products from *C. papaya* interacts with different residues of dengue virus protein having favourable binding energy and suitable drug likeness. It was observed that apigenin and luteolin favourable binding energy (-7.7 Kcal/mol) but luteolin may be suitable lead compound due to inhibitory effect on target receptor as well as ADME efficacy through pharmacokinetics evaluation. In conclusion, it was obtained through faster screening by using software that phytoconstitent luteolin from *C. papaya* may use future drug as lead compound for the prevention of dengue fever. It is suggested that functional assay (*in vivo* and *in vitro* assay) should be carried out for the validation of present predictive data.

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***Keywords*:** Molecular docking, *Carica papaya*, Phytochemicals, Dengue virus protein, Preotein-ligand binding, Pharmacokinetics

1. INTRODUCTION

The Culex mosquito-transmitted Dengue virus (DENV) under genus Flavivirus, a member of Flaviviridae family, cause dengue fever worldwide, also in many parts of India. In recent research, vaccines and/or therapies found for this vector-borne disease (Recker et al., 2016; Low et al., 2017; Srivarangkul et al., 2018) and great epidemiological issues with a national and international importance (Guzman et al., 2010). It was reported that dengue virus has four serotypes DENV-1, DENV-2, DENV-3 and DENV-4 having structural and sequence similarities (Elahi et al., 2014; Mirza et al., 2016). Each serotype has different interactions with antibodies in human blood serum (Mukhopadhyay, et al., 2005; Luo et al., 2008; Guzman et al., 2010). It was also observed by other researchers that the viral genome is translated into three structural proteins such as capsid (C), pre-membrane (prM), envelope (E) and seven types viz. NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 non-structural proteins (Pereira et al., 2008; Rothwell et al., 2009; Deng et al., 2012; Suganya and Mahendran, 2016).

Generally, NS2B-NS3 protease is a combination of two dengue viral proteins as 2B and 3 and these two proteins in complex replicate faster during dengue fever. NS2B cofactor was essential for the protease catalytic activity of NS3 (de Almeida et al., 2013). This protein complex is very important for the recent research on anti-dengue drug development. Till date several small molecules against DENV NS2B/NS3 protease showed less success in case of *in silico* study and confusing results have obtained in drug development (Schüller et al., 2011; de Almeida et al., 2013).

Carica papaya Linn. is tree under family caricaceae having potent medicinal properties for prevention of several diseases (Aravind et al., 2013). The leaf contains carpaine, is a major alkaloid, phenolic compounds viz. protocatechuic acid, p-coumaric acid, caffeic acid, chlorogenic acid, kaempferol, and 5,7dimethoxycoumarin and flavonoids such as myricetin, quercetin, kaempferol, luteolin, and apigenin of *Carica papaya* (Miean and Mohamed, 2001; Canini et al., 2007; Lim, 2012; Senthilvel et al., 2013; Yogiraj et al., 2014). On the other hand, only flavonoids viz. quercetin and kaempferol were detected in the shoot of *Carica papaya* (Miean and Mohamed, 2001). The leaf extract of *C. papaya* is well known to prevent dengue fever in human and decreased the value of platelets count, leucocytes count, and neutrophils count in blood (Ahmad et al., 2011).

In silico study with special reference to molecular docking determines easy screening of receptor-ligand binding position and interaction with residues to know potent lead in the drug development process (Lionta et al., 2014; Kontoyianni, 2017). It was known that the lead compound(s) is whether effector or inhibitor for each receptor. The pharmacokinetic and toxicological profiles support the bioactivity of the compound(s) as drug likeness (Vyas et al., 2008; López-Vallejo et al., 2011; Lipinski et al., 2012; Ferreira et al., 2015; Daina et al., 2017).

The present study was attempted to detect inhibitory potential among established phytochemicals especially flavonoids present in leaf of *C. papaya* against the protein (NS2B/NS3 protease) of dengue virus through molecular docking and pharmacokinetic study for evaluating suitable lead(s) for antidengue drug development.

2. MATERIALS AND METHODS

2. 1. Receptor selection

The three-dimensional (3-D) structure of this protease associated with NS2B cofactor available in the European protein data bank (PDB). The 3-D structure of DENV-2 as NS2B/NS3 protease was retrieved from the protein data bank (http://www.ebi.ac.uk/pdbe/), PDB ID: 2FOM (Erbel et al., 2006). There are 46 numbers and 151 numbers of residues found in chain A and B respectively. All water molecules were removed and on the final stage hydrogen atoms were added to the target protein molecule in AutoDock Tool (Morris et al., 1998). The three-dimensional (3-D) crystal structure of NS2B-NS3 protease is exhibited in Figure 1.

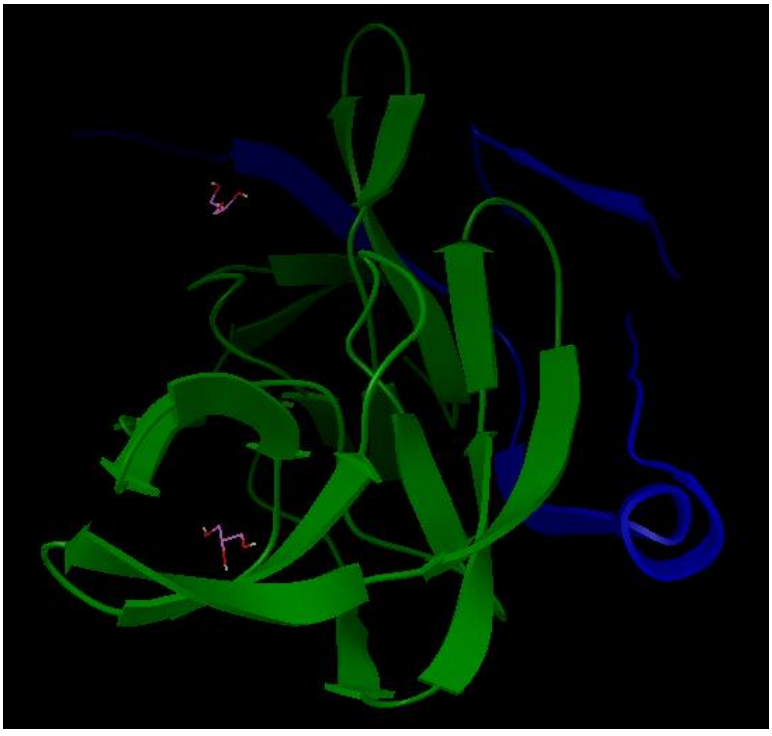


Figure 1. Crystal structure of dengue virus protein NS2B/NS3 protease
[(subunit A = blue colour (NS2B) and B = green colour (NS3 protease) as ribbon structure;
GOL = line structure in CPK)]

2. 2. Phytoligands selection

Established 10 phytocompounds especially flavonoids from *Carica papaya* Linn. were selected from the previous report (Canini et al., 2007). The information of ten phytoligands was taken from PubChem database (http://www.ncbi.nlm.nih.gov/ pccompound/) and converted into 3-D structure by using CORINA online server (http://www.mol-net.de) after incorporating the canonical SMILES string for each phytochemical and the structures of the ligands are depicted in Figure 2.

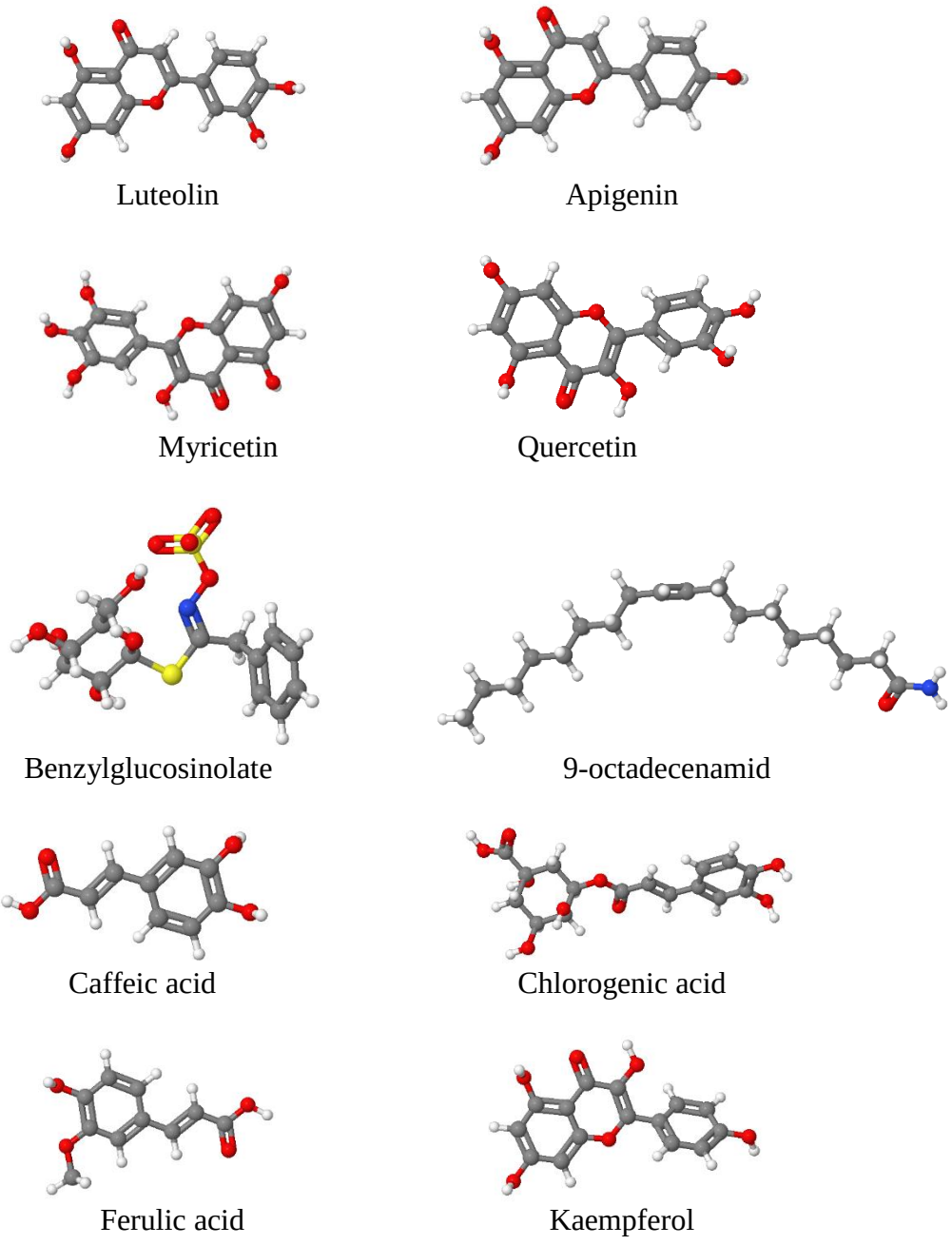


Figure 2. Three-dimensional structure of ligands as established phytochemicals from *Carica papaya* Linn.

2. 3. Molecular docking study

The molecular docking was done through PyRx software (Version 0.8) developed by Trott and Olson (2010). The molecular docking result for each compound was visualized as output .pdbqt file by using molecular graphics laboratory (MGL) tool, developed by Morris et al., (1998). The PyRx software is an easy virtual screening with minimum steps and time to

obtain docking output file. It is a non-commercial, less time-consuming docking tool that basically predict receptor-ligand binding along with providing energy value for each test compound. Docking of 9 phytochemicals with NS2B/NS3 protease (PDB ID: 2fom) was analysed for the docking of phytoligands and the NS2B/NS3 protease (receptor) to identify the residues involved in the study of receptor-ligand interactions. All the ligands and receptor file were individually taken prior converted to pdbqt format. The 3-D interaction of the individual phytochemical against NS2B/NS3 protease was finally visualized by using MGL tool, to determine some specific contacts between the atoms of the test compounds and amino acids of the studied receptor.

2. 4. Pharmacokinetics study

Pharmacokinetic study for each phytochemical was done using the online tool ADMET-SAR (absorption, distribution, metabolism, excretion, toxicity – structure activity relationship) to check whether the compound has fulfilled the conditions as drug candidate (Cheng et al., 2012).

3. RESULTS AND DISCUSSION

The docking results clearly indicated that the interaction of two phytochemicals (ligands) present in the leaf of *Carica papaya* Linn. against NS2B/NS3 protease (receptor), data were energetically favourable. The active site of the studied receptor is exhibited in Figure 3.

It was observed from Table 1 that the low energy value (kcal/mol) was obtained in apigenin and luteolin (-7.7) followed by myricetin (-7.5), quercetin and benzylglucosinolate (-7.4), chlorogenic acid (-7.3), kaempferol (-7.1) but caffeic acid (-6.0) and ferulic acid (-5.9) were showed close value while high value obtained in 9-octadecenamide (-5.0). It was found that the hydrophobic interactions connected with amino acids Glu89, Glu90, Lys104, Pro72 for apigenin, Asp75, Gly153, Ser131, Leu128, Phe130 for luteolin, Gly153, Tyr161, His51, Trp50 for myricetin, Gly151, Pro132, Ser131, Leu128, His51 for quercetin, Tyr161, Leu128, Asn152 for benzylglucosinolate, Val72, Val154, Val155, Thr118, Trp50, His51 for chlorogenic acid, Leu74, Pro72, Lys104, Asn105 for kaempferol, Val155, Val154, Thr118 for caffeic acid, Gly153, Val154, Asn119, Thr118 for ferulic acid and Tyr161, Leu128, Gly151, Gly153, His51, Pro132 for 9-octadecenamide (Table 1).

All the phytoconstituents were found hydrophobic in nature. The 3-D ribbon structure for binding pose for two phytoligands was obtained through PyRx software and the 3-D interaction structure of two ligands procured through MGL Tool interface (Moris et al., 1998) and energetically favourable compounds is depicted Figure 4 (A–B). In Figure 4 (a–b), the 3-D binding interaction with residues for each compound is exhibited.

The data were obtained during interaction study that hydrogen bonding formed 2 nos. for apigenin, luteolin, kaempferol, benzylglucosinolate and ferulic acid, 1 no. for myricetin and 9-octadecenamide, 3 nos. for quercetin, 4 nos. for caffeic acid and 6 nos. for chlorogenic acid, respectively with NS2B-NS3 protease. The hydrogen bonds were connected with particular residues as Asn105 and Arg107 for apigenin; Tyr150 and unknown for luteolin; Gly153 and Asp75 for benzylglucosinolate; Asn88 and Arg107 for kaempferol; Val155 and Thr120 for ferulic acid; Ser135 for 9-octadecenamide; Gly151 for myricetin; Tyr150, Gly151, Gly153 for

quercetin and Gly153, Lys73, Thr118, Asn119 for caffeic acid; Asn152, Gly153, Asn119, Thr120 for chlorogenic acid respectively (Table 1 and Figure 4).

The phytoligands apigenin and luteolin showed highest binding affinity and lowest energy value (-7.7 Kcal/mol) and two numbers of hydrogen bonding but it was observed only luteolin found near catalytic triad connected with residue ASP75 while benzylglucosinolate shwed poor binding energy value but same numbers of hydrogen bonding connected with two residues of active site (Asp75 and His51). Dengue fever is an epidemic issue worldwide. The target receptor in the present prediction is the viral protein NS2B/NS3 protease is mainly targeting for drug development on anti-dengue infection as per earlier study (Wu et al., 2015). This active site inhibition might be considered as lead compounds. The present results indicated combinations of phytochemicals in *C. papaya* leaf may inhibit the multiplication of viral activity, that is the reseaon may phytochemicals in combination help in anti-dengue natural product when patients take leaf juice of *C. papaya* during dengue fever. Till date, few synthetic drugs such as Ivermectin, selamactin, methylbenzethonium chloride, tyrothricin and alexidine hydrochloride have been reported for DENV2 NS3/NS2B protease inhibitors (Oliveira, et al., 2014).

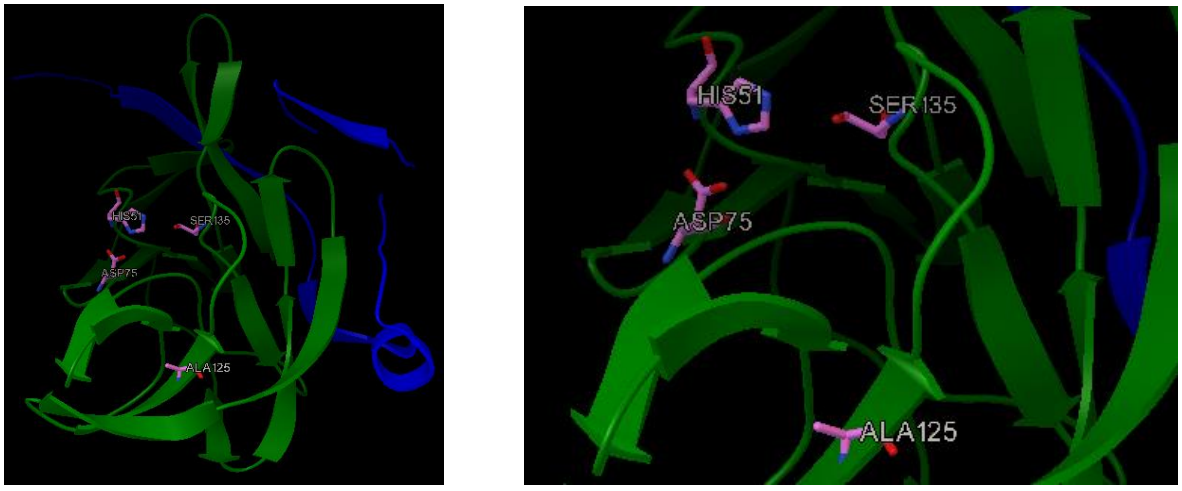


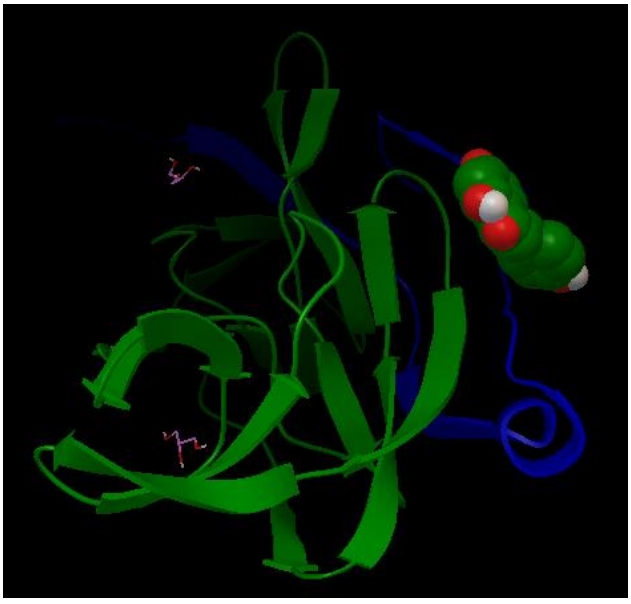
Figure 3. Ribbon representation of central and catalytic triad in the active site of NS2B/NS3 protease (Left = normal; Right = enlarge view)

Major reports are targeting NS2B-NS3 protease by *C. papaya* leaf small-molecule inhibitors have been found in the active site but natural products have been discovered prevention of dengue infection (Mucsi and Pragai, 1985; Chappell et al., 2008; Ahmad et al., 2011; Zandi et al., 2011; Kim et al., 2013; Senthilvel et al., 2013; Yogiraj et al., 2014; Wu et al. 2015; Mishra, 2016). Moreover, Wu et al. (2015) have investigated allosteric inhibition site for small-molecules in drug designing against dengue virus (NS2B-NS3 protease) both in predictive as well as *in vitro* experimental study. In other study against NS2B-NS3 protease inhibition, quercetin compound showed suitable inhibitor in experiment and *in silico* study in relation to binding energy, hydrogen bonding etc. (Mucsi and Pragai, 1985; Ahmad et al., 2011; Zandi et al., 2011; Senthilvel et al., 2013; Wu et al., 2015). Yildiz et al. (2013) have documented that the allosteric sensitive site of NS2B/NS3 protease is centrally located at Ala125.

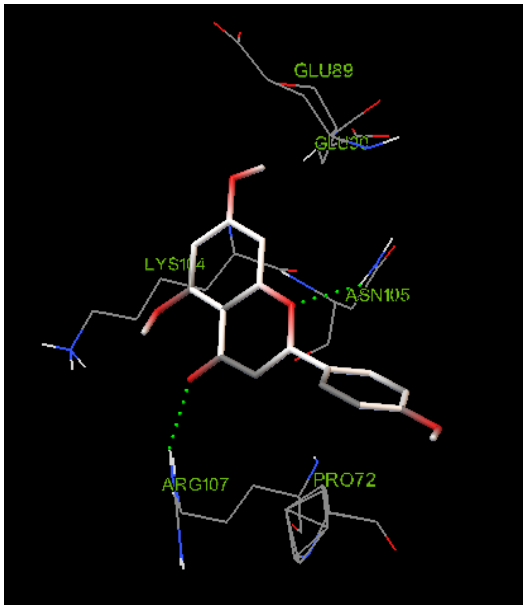
In their study, it was also observed a catalytic triad (His51 – Asp75 – Ser135) in the active site and same active site also was mentioned by other researchers (Schöne et al., 2017; Silva et al., 2018**)**. According to Mishra (2016), the amino acid residues such as Met49, Lys73, Lys74, Leu76, Trp83, Leu85, Glu86, Gly87, Glu88, Trp89, Thr118, Thr120, Ile123, Val146, Val147, Asn152, Val154, Ala164, Ile165, Ala166 and Asn167 were reported in the binding pocket of NS2B/NS3 protease receptor.

Table 1. Molecular docking analysis for leaf phytochemicals of *C. papaya* against dengue virus protein (NS2B/NS3 protease).

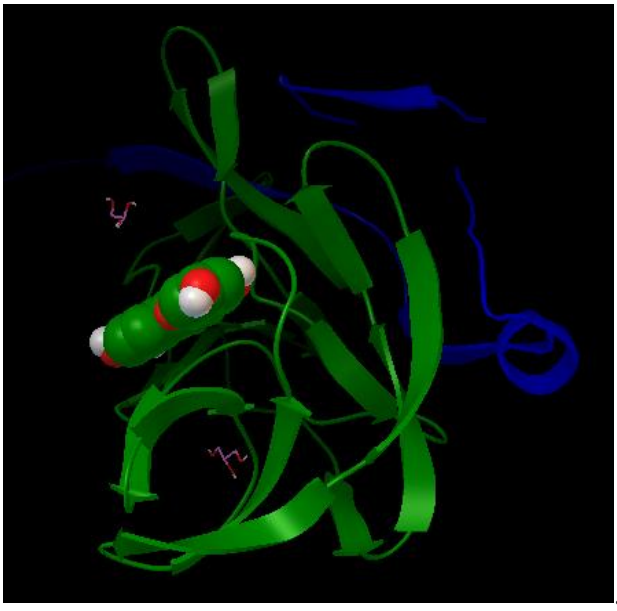
Sl. No.	**Ligands**	**Binding energy (kcal/mol)**	**Interacting amino acids (hydrophobic interaction)**	**Hydrogen bond nos. & contact residues**
1.	Apigenin	-7.7	Glu89, Glu90, Lys104 & Pro72	2 & Asn105 & Arg107
2.	Luteolin	-7.7	Asp75, Gly153, Ser131, Leu128 & Phe130	2& Tyr150 & unknown
3.	Myricetin	-7.5	Gly153, Tyr161, His51, Arg54, Trp50, Val72 & Asp75	1 & Gly151
4.	Quercetin	-7.4	Pro132, Ser135, Leu128 & His51	3& Tyr150, Gly151 & Gly153
5.	Benzylglucosinolate	-7.4	His51, Tyr161, Gly151 & Leu128	2& Gly153 & Asp75
6.	Chlorogenic acid	-7.3	Val72, Val154, Val155, Thr118, Trp50, His51	6 & Asn152, Gly153, Asn119 & Thr120
7.	Kaempferol	-7.1	Leu74, Pro72, Lys104, Asn105	2& Asn88 & Arg107
8.	Caffeic acid	-6.0	Val155, Val154, Thr118,	4 & Gly153, Lys73, Thr118 & Asn119
9.	Ferulic acid	-5.9	Gly153, Val154, Asn119, Thr118	2 & Val155 & Thr120
10.	9-octadecenamide	-5.0	Tyr161, Leu128, Gly151, Gly153, His51, Pro132	1 & Ser135



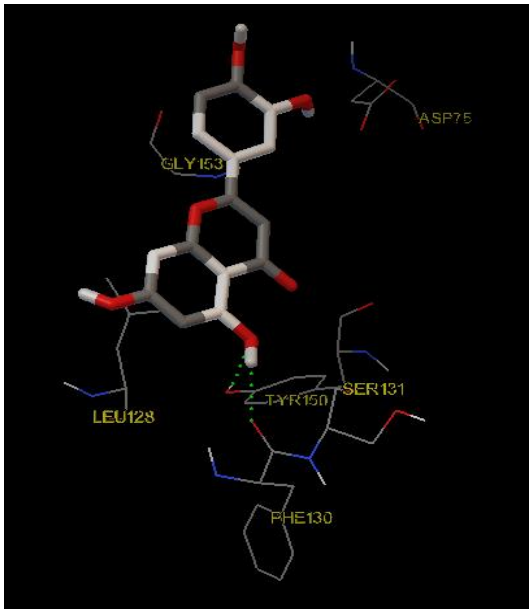
A. apigenin binding pose



a. binding interaction



B. luteolin binding pose



b. binding interaction

Figure 4. Three-dimensional structure of protein-ligands docking pose (A-B) and binding interaction (a-b)

Another part of results indicated pharmacokinetics through "Lipinski's Rule Five" (Lipinski et al., 2012), to determine molecular properties and drug likeness of selected active compounds found in *Carica papaya* leaf. It was known from the rule that molecular properties

are important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion and also toxicity study through ADMET prediction (Lipinski et al., 2012). It was established that the drug molecules have poor absorption and permeation, when compounds have molecular weight >500 and logP >5 as well as number of hydrogen bond donors (NH and OH) and acceptors (O and N atoms) for the compounds should be within Lipinski's rule five, as stated <5 for donors and <10 for acceptors (Senthilvel et al., 2013; Paramashivam et al., 2015). The ADMET properties prediction on docked compounds revealed that six compounds were observed for Blood-Brain barrier (BBB) penetration, nine compounds for human intestinal absorption and seven compounds for Caco2 permeability were observed. All compounds were found P-glycoprotein non-inhibitor and kaempferol was substrate inhibitor and luteolin, quercetin and myricetin were showed substrate and rest compounds were non-substrate for P-glycoprotein.

Table 2. ADMET-Prediction profiles for phytochemicals of *C. papaya* leaf

Absorption							**Distribution**
Sl. No.	**Phytoligands**	**Blood-brain barrier**	**Caco-2 permeability**	**Human intestinal absorption**	**P-glycoprotein inhibitor**	**P-glycoprotein substrate**	**Subcellular localization**
1.	Apigenin	BBB+	Caco2+	HIA+	NI	NS	Mitochondria
2.	Luteolin	BBB-	Caco2+	HIA+	NI	S	Mitochondria
3.	9-octadecenamide	BBB+	Caco2+	HIA+	NI	NS	Lysosome
4.	Ferulic acid	BBB-	Caco2+	HIA+	NI	NS	Mitochondria
5.	Caffeic acid	BBB-	Caco2+	HIA+	NI	NS	Mitochondria
6.	Kaempferol	BBB+	Caco2-	HIA+	NI	SI	Mitochondria
7.	Chlorogenic acid	BBB+	Caco2-	HIA+	NI	NS	Mitochondria
8.	Benzylglucosinolate	BBB+	Caco2+	HIA+	NI	NS	Mitochondria
9.	Quercetin	BBB+	Caco2-	HIA+	NI	S	Mitochondria
10.	Myricetin	BBB-	Caco2+	HIA-	NI	S	Mitochondria

Metabolism								**Excretion**
Sl. No.	**Phytoligands**	**CYP450 2C9 inhibitor**	**CYP450 2C9 substrate**	**CYP450 2D6 inhibitor**	**CYP450 2D6 substrate**	**CYP450 3A4 inhibitor**	**CYP450 3A4 substrate**	**ROCT**
1.	Apigenin	I	NS	NI	NS	I	NS	NI
2.	Luteolin	NI	NS	NI	NS	I	NS	NI
3.	9-octadecenamide	NI	NS	NI	NS	NI	NS	NI
4.	Ferulic acid	NI	NS	NI	NS	NI	NS	NI

5.	Caffeic acid	NI	NS	NI	NS	NI	NS	NI
6.	Kaempferol	I	NS	NI	NS	I	NS	NI
7.	Chlorogenic acid	NI	NS	NI	NS	NI	NS	NI
8.	Benzylglucosinolate	NI	NS	NI	NS	NI	S	NI
9.	Quercetin	NI	NS	NI	NS	I	NS	NI
10.	Myricetin	NI	NS	NI	NS	I	NS	NI

Toxicity

Sl. No.	**Phytoligands**	**Acute oral toxicity**	**Fish toxicity**	**Honey bee toxicity**	**AMES toxicity**	**Carcinogens**
1.	Apigenin	III	HFHMT	HHBT	NT	NC
2.	Luteolin	II	HFHMT	HHBT	NT	NC
3.	9-octadecenamide	III	HFHMT	LHBT	NT	NC
4.	Ferulic acid	IV	HFHMT	HHBT	NT	NC
5.	Caffeic acid	IV	HFHMT	HHBT	NT	NC
6.	Kaempferol	II	HFHMT	HHBT	NT	NC
7.	Chlorogenic acid	III	HFHMT	HHBT	NT	NC
8.	Benzylglucosinolate	III	HFHMT	HHBT	NT	NC
9.	Quercetin	II	HFHMT	HHBT	NT	NC
10.	Myricetin	II	HFHMT	HHBT	NT	NC

NI = Non-inhibitor; I = Inhibitor; NS = Non-substrate; S = Substrate; SI = Substrate inhibitor; ROCT = Renal Organic Cation Transporter; I = Category I (LD_{50} values less than or equal to 50 mg/kg); II = Category II (LD_{50} values greater than 50 mg/kg but less than 500 mg/kg); III = Category III (LD_{50} values greater than 500 mg/kg but less than 5000 mg/kg) and IV = Category IV (LD_{50} values greater than 5000 mg/kg); H = High; L = Low; FHMT = Fathead minnow toxicity; HBT = Honey bee toxicity; NT = Non-toxic; NC = Non-carcinogen

For metabolism study, an important parameter is cytochrome P450 (CYP), which is known as isozymes group and it is involved in the metabolism of several compounds viz. drugs, fatty acids, steroids, bile acids and carcinogens (Anitha et al., 2014). Predicting the cytochrome P450 (CYP) analysis, the results for CYP450 2C9 inhibitor, all compounds were found non-inhibitor except apigenin and kaempferol but all compounds were observed non-substrate in case of CYP450 2C9 substrate while all compounds were obtained non-inhibitor and non-substrate for CYP450 2D6 inhibitor and substrate but all compounds were observed non-inhibitor except five compounds viz. apigenin, luteolin, kaempferol, quercetin and myricetin and all compounds were showed non-substrate except one compound namely benzylglucosinolate for CYP450 3A4 inhibitor and substrate. In case of toxicity assessment, results suggested that all compounds were observed non-mutagenic and non-carcinogenic.

The oral LD_{50} values were not observed category I i.e. <50ppm for all compounds but four compounds category II (luteolin, kaempferol, quercetin and myricetin), four compounds category III (apigenin, benzylglucosinolate, chlorogenic acid and 9-octadecenamide) and two compounds category IV (ferulic acid and caffeic acid) were obtained. All the compounds were obtained high fish toxicity while high honey bee toxicity was found in all the compounds except 9-octadecenamide (Table 2).

These parameters have predicted through high throughput and the measurement of values for absorption, metabolism, and toxicity that helps in the detection of active lead compounds at early drug discovery. According to Tsaioun et al. (2009), previously ADME study benefitted in relation to the development of effective lead compounds in drug discovery. In this context, researchers have stated that ADMET properties prediction with special reference to Blood-Brain barrier (BBB) penetration, P-glycoprotein substrate, renal organic cation transporter, human intestinal absorption and Caco2 permeability on docked compounds are suitable pharmacological parameters for drug designing (Alavijeh et al., 2005; Hurst et al., 2007; Senthilvel et al., 2013; Anitha et al. 2014). I

t was also an established fact that the drug likeness of active compounds as therapeutic agents may be detected through oral bioavailability that entangles physico-chemical, physiological and few biopharmaceutical factors (Hurst et al., 2007). In case of metabolism, other important parameters are cytochrome P450 (CYP) of different types, which belongs to isozymes group and it is participated in the metabolism of drugs, fatty acids, steroids, bile acids and carcinogens (Guengerich et al., 2003).

4. CONCLUSIONS

It is concluded that few compounds are observed suitable for inhibitory effect on DNV2 (NS2B/NS3 protease) through *in silico* study with special reference to molecular docking and pharmacokinetic evaluation. However, quercetin compound from the leaf extracts of *C. papaya* has been showed active phytochemical for anti-dengue remedy (Senthilvel et al., 2013) but in present study favourable binding affinity was obtained for apigenin and luteolin while the flavonoid luteolin showed active site binding with target receptor and suitable ADME profile.

On the other hand, Nuri and Ming (2016) have stated that *C. papaya* leaf juice does not have any side effect neither experimental evidence of dengue fever healing. In other words, few reports have found allosteric as well as competitive and non-competitive inhibitory effect by phytochemicals, quercetin from *C. papaya* is one of the suitable example while 4-hydroxypanduratin A, panduratin A from *Boesenbergia rotunda* and flavonoids such as agathisflavone and myricetin (Frimayanti et al., 2011; Senthilvel et al., 2013; Heh et al., 2013; de Sousa et al., 2014; Wu et al., 2015). The present results also found two flavonoids such as apigenin and luteolin as favourable binding energy, hydrogen bonding etc. but luteolin may be a lead compound due to the active site binding and suitable ADMET profile. Therefore, present study is suggesting experimental study with these phytocompound against NS2B/NS3 protease with the validation of the predictive lead for anti-dengue drug.

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References

[1] Recker M, Vannice K, Hombach J. Jit M, Simmons CP, Assessing dengue vaccination impact: Model challenges and future directions. *Vaccine* 34(38) (2016) 4461-4465

[2] Low JGH, Ooi EE, Vasudevan SG, Current status of dengue therapeutics research and development. *J Infect Dis.* 215(Suppl 2) (2017) S96-102

[3] Srivarangkul P, Yuttithamnon W, Suroengrit A, Pankaew S, Hengphasatporn K, Rungrotmongkol T, et al. A novel flavanone derivative inhibits dengue virus fusion and infectivity. *Antiviral Res.* 151 (2018) 27-38

[4] Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: a continuing global threat. *Nat Rev Microbiol.* 8 (2010) S7-16

[5] Elahi M, Islam MM, Noguchi K, Yohda M, Toh H, Kuroda Y, Computational prediction and experimental characterization of a "size switch type repacking" during the evolution of dengue envelope protein domain III (ED3). *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1884(3) (2014) 585-592

[6] Mirza SB, Salmas RE, Fatmi MQ, Durdagi S, Virtual screening of eighteen million compounds against dengue virus: Combined molecular docking and molecular dynamics simulations study. *Journal of Molecular Graphics and Modelling* 66 (2016) 99-107

[7] Mukhopadhyay S, Kuhn RJ, Rossmann MG, A structural perspective of the flavivirus life cycle. *Nat Rev Microbiol.* 3 (2005) 13-22

[8] Luo D, Xu T, Watson RP, Scherer-Becker D, Sampath A, Jahnke W, et al. Insights into RNA unwinding and ATP hydrolysis by the flavivirus NS3 protein. *The EMBO Journal* 27 (2008) 3209-3219

[9] Pereira R, Kunh JR, Structural proteomics of dengue virus. *Curr Opin Microbiol.* 11 (2008) 369

[10] [10] Rothwell C, Lebreton A, Young Ng C, Lim JY, Liu W, Vasudevan S, et al. Cholesterol biosynthesis modulation regulates dengue viral replication. *Virology* 389 (2009) 8-19

[11] Deng J, Li N, Liu H, Zuo Z, Liew OW, Xu W, et al. Discovery of novel small molecule inhibitors of dengue viral NS2B-NS3 protease using virtual screening and scaffold hopping. *J Med Chem.* 55(14) (2012) 6278-6293

[12] Suganya J, Mahendran R, Insilico QSAR and molecular docking studies of selected medicinal plant compounds against NS5 & NS3 protein of dengue virus: a comparative approach. *Int J Pharm Bio Sci.* 7(3) (2016) (B) 1135-1144

[13] de Almeida H, Bastos IMD, Ribeiro BM, Maigret B, Santana JM, New binding site conformations of the Dengue Virus NS3 protease accessed by molecular dynamics simulation. *PLoS ONE* 8(8) (2013) e72402. doi:10.1371/journal.pone.0072402

[14] Schüller A, Yin Z, Brian Chia CS, Doan DNP, Kim H-K et al. (2011). Tripeptide inhibitors of dengue and West Nile virus NS2B-NS3 protease. *Antiviral Res* 92: 96-101

[15] Aravind, G., Bhowmik, D., Duraivel, S, Harish, G. 2013. Traditional and medicinal uses of *Carica papaya. Journal of Medicinal Plants Studies* 1(1): 7-15

[16] Miean, K.H., Mohamed, S., 2001. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *J. Agric. Food Chem.* 49, 3106-3112

[17] Canini A, Alesiani D, D'Arcangelo G, Tagliatesta P, Gas chromatography-mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. *J Food Compos Anal.* 20 (2007) 584-590

[18] Lim TK, *Carica papaya*. In: Edible medicinal and non-medicinal plants. *Springer* 1 (2012) 693-717

[19] Senthilvel P, Lavanya P, Kumar KM, Swetha R, Anitha P, Bag S, et al. Flavonoid from *Carica papaya* inhibits NS2B-NS3 protease and prevents Dengue 2 viral assembly. *Bioinformation* 9(18) (2013) 889-895

[20] Yogiraj V, Goyal PK, Chauhan CS, Goyal A, Vyas B, *Carica papaya* Linn: An overview. *International Journal of Herbal Medicine* 2(5) (2014) 01-08

[21] Ahmad N, Fazal H, Ayaz M, Abbasi BH, Mohammad I, Fazal L, Dengue fever treatment with *Carica papaya* leaves extracts. *Asian Pac J Trop Biomed.* 1(4) (2011) 330-333

[22] Lionta E, Spyrou G, Vassilatis DK, Cournia Z. Structure-based virtual screening for drug discovery: Principles, applications and recent advances. Curr Top Med Chem. 2014; 14:1923-1938

[23] Kontoyianni M. Docking and virtual screening in drug discovery. In: Lazar I, Kontoyianni M, Lazar A. (editors). Proteomics for drug discovery: Methods in molecular biology. Vol. 1647 New York: Humana Press, 255-266 (2017)

[24] Vyas, V., Jain, A., Jain, A. and Gupta, A. Virtual Screening: A Fast Tool for Drug Design. *Sci Pharm.* 2008; 76: 333-360

[25] López-Vallejo F, Caulfield T, Martínez-Mayorga K, Giulianotti MA, Houghten RA, Nefzi A, et al. Integrating virtual screening and combinatorial chemistry for accelerated drug discovery. *Comb Chem High Throughput Screen.* 14 (2011) 475-487

[26] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev.* 46 (1-3) (2012) 3-26

[27] Ferreira LG, dos Santos RN, Oliva G, Andricopulo AD, Molecular docking and structure-based drug design strategies. *Molecules* 20 (2015) 13384-13421

[28] Daina A, Michielin O, Zoete V, SwissADME: a free web tool to evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules. *Sci Rep.* 7 (2017) 42717. doi: 10.1038/srep42717

[29] Erbel P, Schiering N, D'Arcy A, Renatus M, Kroemer M, Lim SP, et al. Structural basis for the activation of flaviviral NS3 proteases from dengue and West Nile virus. *Nat Struct Mol Biol.* 13 (2006) 372-373

[30] Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J Comput Chem.* 19 (1998) 1639-1662

[31] Trott O, Olson AJ, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput Chem.* 31 (2010) 455-461

[32] Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, et al. SAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *J Chem Inf Model.* 52 (11) (2012) 3099-3105

[33] Wu H, Bock S, Snitko M, Berger T, Weidner T, Holloway S, et al. Novel dengue virus NS2B/NS3 protease inhibitors. Antimicrob Agents Chemother 59 (2015) 1100-1109

[34] de Oliveira SA, da Silva ML, Flávia Oliveira AFCS, da Silva CC, Teixeira RR, De Paula SO, NS3 and NS5 proteins: Important targets for anti-dengue drug design. *Journal of the Brazilian Chemical Society* 25(10) (2014). 1759-1769

[35] Mucsi I, Pragai BM, Inhibition of virus multiplication and alteration of cyclic AMP level in cell cultures by flavonoids. *Experientia* 41 (1985) 930-931

[36] Chappell KJ, Stoermer MJ, Fairlie DP, Young PR, West Nile Virus NS2B/NS3 protease as an antiviral target. *Current Medicinal Chemistry* 15 (2008) 2771-2784

[37] Zandi K, Teoh B-T, Sam S-S, Wong P-F, Mustafa MR, AbuBakar S, Antiviral activity of four types of bioflavonoid against dengue virus type-2. *Virology Journal* 8 (2011) 560. doi:10.1186/1743-422X-8-560

[38] Kim YM, Gayen S, Kang C-B, Joy J, Huang Q, Chen AS, et al. NMR Analysis of a novel enzymatically active unlinked Dengue NS2B-NS3 protease complex. *The Journal of Biological Chemistry* 288 (2013) 12891-12900

[39] Mishra P, Evaluation of Phytochemical compounds from Carica papaya as Potential drugs against Dengue virus: An In silico approach. MSc. Thesis, Department of Bioinformatics Centre for post graduate studies Orissa University of Agriculture and Technology, Bhubaneswar, Odisha (2016).

[40] Yildiz M, Ghosh S, Bell JA, Sherman W, Hardy JA, Allosteric inhibition of the NS2B-NS3 protease from dengue virus. *ACS Chem Biol.* 8(12) (2013) 2744-2752

[41] Schöne T, Grimm LL, Sakai N, Zhang L, Hilgenfeld R, Peters T. STD-NMR experiments identify a structural motif with novel second-site activity against West Nile virus NS2B-NS3 protease. *Antiviral Res.* 146 (2017) 174-183

[42] Silva-Junior E, Schirmeister T, Araújo-Júnior J, Recent advances in inhibitors of flavivirus NS2B-NS3 protease from Dengue, Zika, and West Nile viruses. In: Vector-Borne Diseases and Treatment. Chapter 1 (2018).

[43] Paramashivam SK, Elayaperumal K, Natarajan BB, Ramamoorthy MD, Balasubramanian S, Dhiraviam KN, In silico pharmacokinetic and molecular docking studies of small molecules derived from *Indigofera aspalathoides* Vahl targeting receptor tyrosine kinases. *Bioinformation* 11(2) (2015) 073-084

[44] Anitha, P, Lavanya P, Anbarasu A, Ramaiah S, Molecular docking study of catechins compounds from *Camellia sinensis* against UPPS in *Staphylococcus aureus*. *International Journal for Computational Biology* 3(2) (2014) 03-09

[45] Tsaioun K, Bottlaender M, Mabondzo A, Alzheimer's Drug Discovery Foundation. ADDME-Avoiding drug development mistakes early: central nervous system drug discovery perspective. *BMC Neurol.* 9(1) (2009) S1

[46] Alavijeh MS, Chishty M, Qaiser MZ, Palmer AM, Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery. *NeuroRx* 2(4) (2005) 554-557

[47] Hurst S, Loi CM, Brodfuehrer J, El-Kattan A, Impact of physiological, physicochemical and biopharmaceutical factors in absorption and metabolism mechanisms on the drug oral bioavailability of rats and humans. *Expert Opin Drug Metab Toxicol.* 3(4) (2007) 469-489

[48] Guengerich FP, Cytochromes P450, drugs, and diseases. *Molecular Intervention* 3 (2003) 194-204

[49] [49] Nuri THM, Ming LC, Papaya leaves juice as an alternative treatment for dengue fever. *J App Pharm Sci.* 6(03) (2016) 172-173

[50] Frimayanti N, Chee CF, Zain SM, Rahman NA, Design of new competitive dengue ns2b/ns3 protease inhibitors - A computational approach. *Int J Mol Sci.* 12 (2011) 1089-1100

[51] Heh CH, Othman R, Buckle MJ, Sharifuddin Y, Yusof R, Rahman NA. Rational discovery of dengue type 2 non-competitive inhibitors. *Chem Biol Drug Des.* 82 (2013) 1-11

[52] de Sousa LR, Wu H, Nebo L, Fernandes JB, da Silva MF, Kiefer W, et al. Flavonoids as noncompetitive inhibitors of Dengue virus NS2B-NS3 protease: inhibition kinetics and docking studies. *Bioorg Med Chem.* 23(3) (2014) 466-470