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Research Article

Molecular docking study of *Lens culinaris* L. phytochemicals to NS3-NS2B protease of dengue virus serotype 2

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Abstract

Forty-three phytochemicals present in *Lens culinaris* were evaluated through *in-silico* molecular docking studies for their binding affinities to the NS2B-NS3 activator-protease complex of dengue virus serotype 2 (DENV-2). Among the various compounds tested, flavonoids (flavanols, flavonols, proanthocyanidins, flavanones, flavones, and anthocyanins) demonstrated high binding affinities for the protease complex. Eriodictyol-7-O-rutinoside showed the least predicted binding energy at -9.1 kcal/mol followed by luteolin-7-O-glucoside at -8.8 kcal/mol. Glycosidic linkages appeared to enhance the binding affinities of flavonoids, aldohexoses being more potent than aldopentoses. Besides flavonoids, other classes of compounds demonstrating high binding affinities for the protease were carotenoids, phytosterols, and polyphenolic compounds like resveratrol and trans-resveratrol 3-O-b-glucoside (piceid), the latter showing predicted binding energy of -8.5 kcal/mol versus predicted binding energy of -7.2 kcal/mol for resveratrol. The 2D interactions of four high binding affinity compounds like eriodictyol, eriodictyol-7-O-rutinoside, catechin gallate, and luteolin-7-O-glucoside showed that all four compounds bound to the active site of the NS3 protease and not to the activator NS2B. Lys74 of NS3 was the common amino acid interacting with all four phytochemicals. Analysis of physicochemical properties of the compounds (Lipinski's Rule of 5) showed that the high binding affinity compounds have less than two violations, indicating that they can serve as useful lead compounds or as dengue virus serotype 2 therapeutics.

Keywords: Dengue, *Flaviviridae*, *Lens culinaris*, Phytochemical, Flavonoids

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Introduction

Dengue is a hemorrhagic fever caused by four serotypes of dengue virus DENV-1, DENV-2, DENV-3, and DENV-4 (Mishra et al., 2011; Ab-Fatah et al., 2015). The various serotypes belonging to the *Flaviviridae* family share about 65% genetic similarities; a fifth serotype has also been discovered (DENV-5) (Mustafa et al., 2015). Dengue is transmitted by female mosquitoes, mainly of the *Aedes aegypti* and also the *Aedes albopictus* type (Carrington and Simmons, 2014). According to the World Health Organization (WHO), Dengue can be sub-clinical to more severe flu-like symptoms. Some people may develop severe dengue complications ranging from severe bleeding, organ impairment and/or plasma leakage (World Health Organization, 2021).

It has been estimated from modeling studies that 3.9 billion people worldwide are at risk from dengue viruses in 129 countries globally, with 70% of the dengue burden falling in Asian countries (Brady et al., 2012; Bhatt et al., 2013). According to the WHO, 505,430 cases of dengue were reported in 2000, over 2.4 million in 2010, and 5.2 million in 2019 (World Health Organization, 2021). The year 2020 witnessed increased dengue infections in Bangladesh, Brazil, Cook Islands, Ecuador, India, Indonesia, Maldives, Mauritania, Mayotte (Fr), Nepal, Singapore, Sri Lanka, Sudan, Thailand, Timor-Leste, and

Yemen (https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengu). The ongoing Coronavirus disease (COVID-19) pandemic can only add to the heavy burden of dengue in those countries. The disease occur primarily from the onset of summer rains due to the breeding of both *Aedes* species that occur only in the clear water of leftover containers outside homes containing flower pots or car tires (Adebote et al., 2011). It is therefore of paramount importance not only to control dengue through controlling *Aedes* spp. mosquitoes, but also to discover suitable therapeutics for this disease.

According to WHO, there is no treatment for dengue fever. Treatment is mainly symptomatic by administering acetaminophen or paracetamol for pain and fever and transfusion of platelet if thrombocytopenia develops (Chairulfatah et al., 2003). Currently, only a live attenuated vaccine "CYD-TDV" against dengue and chimeric yellow fever 17D, is approved and available in several countries. However, there are ongoing attempts to produce vaccines against DENV using a multi-pronged approach of live attenuated vaccine, inactivated vaccine, recombinant subunit vaccine, viral vectored vaccine, and DNA vaccine (Yauch and Shresta, 2014). For the CYD-TDV vaccine, WHO has recommended its use only in dengue epidemic areas (World Health Organization, 2016).

The lack of suitable vaccines or other therapeutics against a rising mosquito-transmitted viral disease is now compelling scientists to look for other alternatives. The most popular alternative is to identify inhibitors against vital viral proteins that are responsible for the viral function or replication. Such identification is made through *in-silico* studies involving molecular docking methods against the non-structural protein (NS2B-NS3) as one of the major targets of DENV (Rothan et al., 2012). However, it is not the only target protein used by scientists. The NS3 protein is a serine protease of the dengue virus, in complex with the activator protein NS2B, catalyzes the viral polyprotein processing at different sites. Therefore, it plays a vital role in inhibiting the dengue virus's maturation (Katzenmeier, 2004). Other target proteins, for instance, include NS4B (Paul et al., 2016) and NS5 methyltransferase (Kausar et al., 2019), which have also been used in molecular docking studies towards identifying possible inhibitors of DENVs as lead or therapeutic compounds.

Lens culinaris Medik (*Fabaceae*) seeds are prized lentils in Bangladesh, India, and Pakistan, where the soup of seeds is consumed regularly. In a previous molecular docking study, we have shown that several phytochemicals from *Lens culinaris* have high binding affinities for the main protease (Mpro) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Hasan et al., 2020). Therefore, the objective of the current study was to evaluate the binding affinities of the previously studied 43 phytochemicals from *Lens culinaris* to NS2B-NS3, as an activator-protease complex of DENV serotype 2.

Materials and Methods

Three-dimensional structure of Dengue protease (NS2B-NS3 protein complex)

The Protein Data Bank file (pdb) file (2FOM) of NS2B-NS3 of DENV-2 was used as published by Erbel et al. (2006). The protein consists of two chains and 185 residues length with a resolution of 1.5 $\AA$ (Sarwar et al., 2018). The central hydrophilic region of NS2B (NS2B; residues 49 to 95) is required by NS3 protease to perform proteolysis activity and stabilize folding. As a result, the hydrophilic domain of NS2B interacts with NS3 protease and forms a fully active site (Yang et al., 2011). Previous functional profiling studies have shown that the NS2B-NS3 proteases of the 4 DENV serotypes share similar peptide-substrate structure activity relationships using tetrapeptide and octapeptide substrate libraries (Li et al., 2005). Hence, any functional inhibitor of NS2B-NS3 must be capable of inhibiting all four DENV serotypes. A monomeric form of the protein was used for molecular docking.

Compounds used in docking studies

We have studied 43 phytochemicals known to occur in *Lens culinaris* (Ganesan and Xu, 2017). Ligand molecules were downloaded from Pubchem (Ihlenfeldt, 2018) as structure data file (sdf) format (Isa et al., 2020). They were optimized with the force field type MMFF94 using Openbable softwares and saved as protein data bank format file plus charges (q) and Autodock atom types (t) format (PDBQT).

Ligand molecular docking studies)

A blind molecular docking was using AutoDock Vina software (Trott and Olson, 2010). We reported the binding energy values (ΔG) as an average of the top values from those docking programs. The more negative ΔG values, the better is the binding affinity of the ligand to the target. We have used exhaustiveness 16 for docking. The grid box in Autodock Vina was generated targeting the entire protein, where the center was at X: -1.109, Y: -15.675 & Z: 17.074, and the dimensions of the grid box were X: 58.77, Y: 61.24 & Z: 49.18 (unit of the dimensions, $\AA$). The figures show the pose of selected phytochemicals bound to dengue protease as obtained from PyMOL and displayed using Discovery Studio 4.1 (Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 4.5, 2015). The poseview tool in the ProteinPlus server was used to obtain the 2D diagram of interaction between protein and ligands (Stierand and Rarey, 2010).

Lipinski's rule of five (Ro5)

Lipinski's rule of 5 or Ro5 was followed to determine the drug like properties of the phytochemicals of *Lens culinaris* in the present work (Giménez et al., 2010; Fernandes et al., 2016). The rule states that molecules, which are poorly absorbed by the intestinal wall (i.e., poor oral bioavailability) would present any two or more of these characteristics: molecular weight more than 500, lipophilicity (log P>5), hydrogen-bond (HB) donor groups (expressed as the sum of OHs and NHs groups) more than 5, more than 10 HB acceptor groups

(defined as the sum of Os and Ns atoms), and molar refractivity outside a range of 40-130.

Biological activity prediction (ADME analysis)

The phytochemicals were evaluated for their potential bioactivity (ADME or absorption, distribution, metabolism, and excretion) by calculating their biological activity scores in different capacities like G-protein coupled receptor (GPCR) ligands, ion channel modulators, kinase inhibitors, nuclear receptor inhibitors, protease inhibitors, and enzyme inhibitors. The various parameters were evaluated with the aid of the software Molinspiration (www.molinspiration.com, Nova Ulica, Slovensky Grob, Slovak Republic) (Rakib et al., 2020).

Results and Discussion

DENV-2 NS3 protease is a 69kDa protein with a serine protease domain located within the 167 amino acid residues of the N-terminus (Li et al., 1999); other studies have mentioned this N-terminus domain as comprising of amino acid residues 1-185 (Constant et al., 2018). The protease has a cofactor NS2B, and the minimal region of NS2B required for NS3 activation is a hydrophilic region consisting of amino acid residues 47/49-95 (Falgout et al., 1993). The NS2B protein is required for optimal activation of the NS3 protease and to stabilize the folding of the proteins (Yang et al., 2011).

Altogether 43 phytochemicals from *Lens culinaris* were evaluated for their binding affinity to NS2B-NS3 of DENV-2. The phytochemicals can be overall divided into two groups; the flavonoid and the non-flavonoid group of compounds. The flavonoid group is comprised of flavonols, proanthocyanidins, flavones, and anthocyanins. The non-flavonoid group contained compounds, which can be further categorized under several sub-groups like hydroxybenzoic acids, hydroxycinnamic acids, polyphenolic compounds (other than belonging to the flavonoid group), carotenoids, and phytosterols. The binding energy (ΔG), expressed in –kcal/mol, is inversely proportional to the binding affinity of a given phytochemical for the protease complex. The binding energies of the various phytochemicals are shown in Table 1, and their structures are shown in Figure 1.

We performed AutoDock (blind) binding studies with the phytochemicals according to Nguyen et al. (2018). Blind studies do not carry any preconceived notions of the binding target(s) but simply show the result where the substrate (ligand) best binds to the protein. The advantage of blind molecular docking studies is that they can demonstrate other ligand-binding pockets instead of focusing only on the active site. Binding at sites other than the active site can also induce conformational changes in the protein leading to inhibition of protein activity. Therefore, both blind and non-blind docking are considered important tools for computer-simulated drug discovery.

Among the various classes of phytochemicals of *Lens culinaris* studied, except for hydroxybenzoic and hydroxycinnamic acids, the other groups of phytochemicals exhibited in general good binding affinities to NS2B-NS3 protease (Table 1). The flavonoid compounds, irrespective of sub-groups, demonstrated strong binding affinities for NS2B-NS3. The flavanone eriodictyol-7-O-rutinoside exhibited the least predicted binding energy at -9.1 kcal/mol (Table 1). According to PubChem (https://pubchem.ncbi.nlm.nih.gov/), the compound known as eriocitrin, is a disaccharide derivative consisting of eriodictyol substituted by a 6-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl moiety at position 7 via a glycosidic linkage. Eriodictyol itself also showed a comparatively low predicted binding energy at -8.4 kcal/mol. In other instances, in this study, the glycosidic linkage decreases the predicted binding energy and subsequently increases binding affinity. For example, the flavonol quercetin showed predicted binding energy of -8.1 kcal/mol, but quercetin-3-O-glucoside and quercetin-3-O-galactoside showed predicted binding energy of -8.6 kcal/mol and -8.7 kcal/mol, respectively. However, quercetin-3-O-xyloside showed a comparatively higher predicted binding energy at -7.9 kcal/mol, suggesting that aldohexose derivatives are better in decreasing predicted binding energy than aldopentose derivatives. Since the decrease of predicted binding energy makes for increased affinity and can result in a better therapeutic effect, the importance of glycosidic derivatives needs further investigation.

The ability of glycosidic linkages to decrease the predicted binding energy has also been noticed in the present study with flavones (Table 1), where the predicted binding energies for luteolin, luteolin-4-O-glucoside, and luteolin-7-O-glucoside were -8.3, -8.7 kcal/mol, and -8.8 kcal/mol, respectively. In the same way, glycosylated flavonoids were found more bioactive and effective in inhibiting other viral protease targets, such as the main protease (Mpro) of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (Cherrak et al., 2020). Similar results were reported for flavonoids from *Salvadora persica* L. (*Salvadoraceae*) aerial part extracts (Owis et al., 2020). Taken together, the low binding energies demonstrated by the flavonoid group of chemicals call for additional research into their potential as lead compounds in the development of novel medicines against various DENV serotypes. The 2D interactions depicting conventional hydrogen, pi-pi and alkyl bonds of some phytochemicals with NS2B-NS3 DENV-2 protease complex are shown in Figure 2 and Figure 3. We have chosen the 2D interactions with emphasis on phytochemicals giving the lowest predicted binding energies. The 2D diagrams showed that eriodictyol (Figure 2A) and eriodictyol-7-Orutinoside Figure 2B) interact only with the NS2B activator protein-NS3 protease, whereas catechin gallate (Figure 3A) and luteolin-7-O-glucosidewith Figure 3B) interact with the NS2B activator protein-NS3 protease.

The amino acid residues of NS3 interacting with eriodictyol are LysB:74, LeuB:76, TrpB:83, LeuB:85, GlyB:87, ValB:146, LeuB:149, and AsnB:152. As previously reported, the protease domain of NS3 has a catalytic triad made up of His51, Asp75, and Ser135

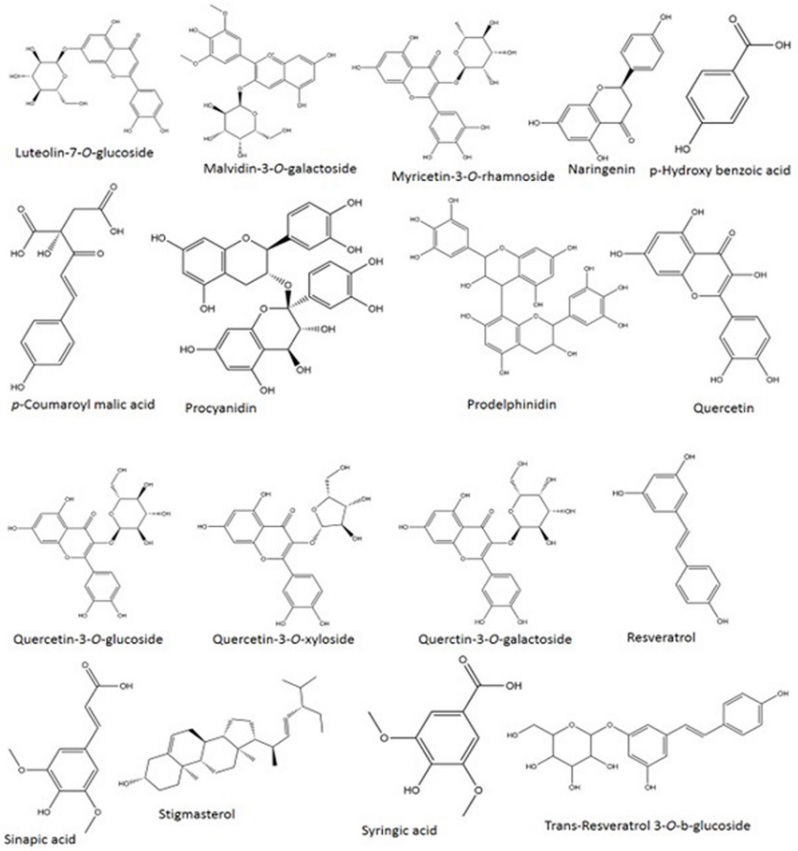

Figure 1: Structures of the various phytochemicals of *Lens culinaris* evaluated in the present study.

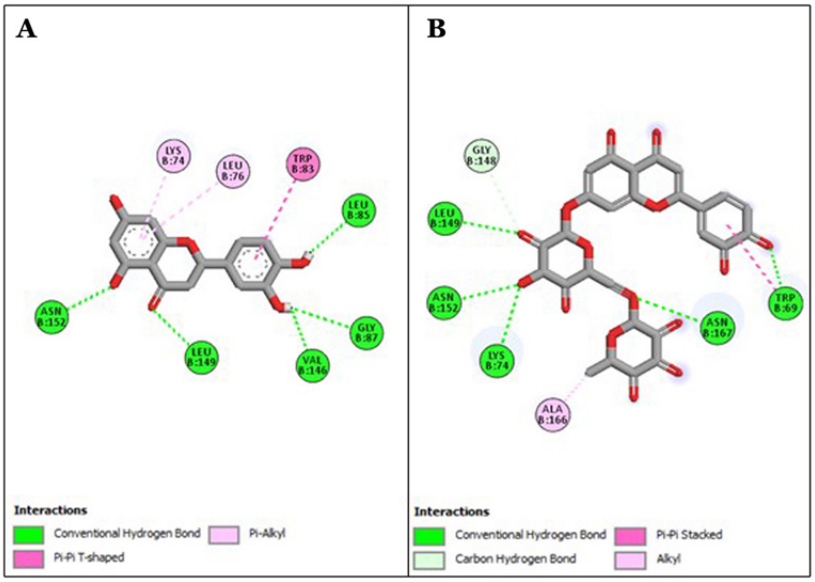

Figure 2: The 2D diagram representations of eriodictyol (A) and eriodictyol-7-O-rutinoside (B) interactions with NS2B-NS3 protease. Eriodictyol and eriodictyol-7-O-rutinoside interact with only the NS3 protease. The interacting amino acids are shown.

Table 1: Dengue Protease (NS2B-NS3) docking result with the phytochemicals of *Lens culinaris*.

	Phytochemicals	**Binding energy (ΔG = kcal/mol)**
Flavonoids		
Flavanols	(-)-Epigallocatechin	-8.1
	(+)-Catechin-3-O-glucose	-7.6
	Catechin	-8.3
	Catechin-7-O-glucoside	-8.1
	Catechin gallate	-8.9
	Epicatechin	-8.2
	Epicatechin gallate	-8.3
Flavonols	Quercetin-3-O-glucoside	-8.6
	Quercetin	-8.1
	Querctin-3-O-galactoside	-8.7
	Quercetin-3-O-xyloside	-7.9
	Kaempferol-4'-O-glucoside	-8.5
	Kaempferol-5-O-glucoside	-8.3
	Kaempferol-3-O-glucoside	-8.4
	Kaempferol-3-O-rutinoside	-7.9
	Myricetin-3-O-rhamnoside	-8.3
	4''''-Acetylsagittatin A	-8.1
Proanthocyanidins	Procyanidin	-8.1
	Prodelphinidin	-8.0
Flavanones	Eriodictyol	-8.4
	Eriodictyol-7-O-rutinoside	-9.1
Flavones	Naringenin	-7.6
	Apigenin	-7.7
	Luteolin	-8.3
	Luteolin-4'-O-glucoside	-8.7
	Luteolin-3',7-diglucoside	-8.0
	Luteolin-7-O-glucoside	-8.8
	5,7-Dimethoxyflavone	-7.4
Anthocyanins	Malvidin-3-O-galactoside	-8.4
Non-Flavonoids		
Hydroxybenzoic acids	Syringic acid	-5.7
	2,3-Dihydroxy benzoic acid	-5.6
	p-Hydroxy benzoic acid	-5.4
	Gallic acid	-5.7
Hydroxycinnamic acids	3-Hydroxy cinnamic acid	-6.1
	p-Coumaroyl malic acid	-6.2
	Sinapic acid	-5.8
Other polyphenols	4-Hydroxy-6-methyl coumarin	-6.3
	Resveratrol	-7.2
	Trans-Resveratrol 3-O-b-glucoside	-8.5
Carotenoids	Lutein	-8.3
Phytosterols	Stigmasterol	-7.4
	Campesterol	-7.3
	β-Sitosterol	-7.1

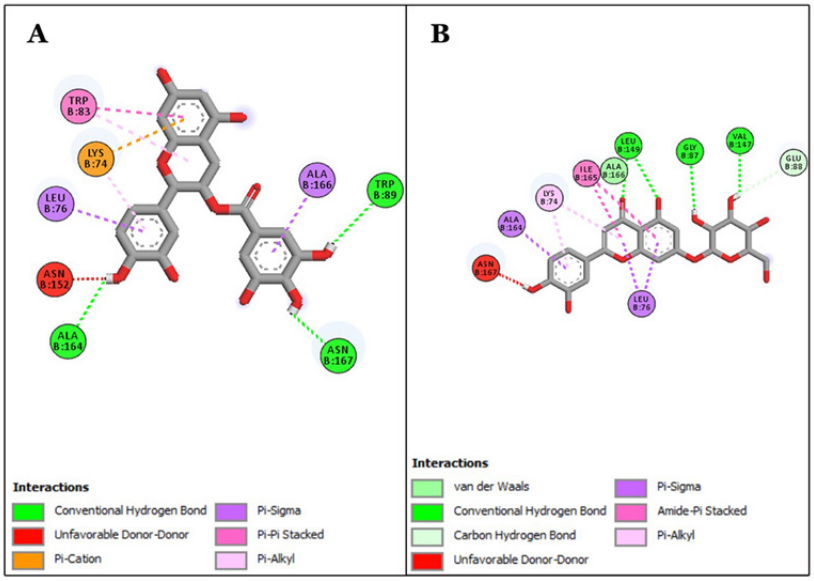

Figure 3: The 2D diagram representations of catechin gallate (A) and luteolin-7-O-glucoside (B) interactions with NS2B-NS3 protease. Catechin gallate and luteolin-7-O-glucoside interacts with only the NS3 protease. The interacting amino acids are shown.

(Yin et al., 2006; Frimayanti et al., 2011). The active site is made up of amino acid residues in S1-S4 sub-pockets like Asp129, Ser135, Tyr150, and Tyr161 in NS3 (S1) pocket; Asp81, Gly82 and Ser83 in NS2B, and Asp75 and Asn152 in NS3 (S2) pocket; Ser85, Ile86 and Lys87 in NS2B (S3) pocket; and Val154 and Ile155 in NS3 (S4) pocket (Hariono et al., 2019). Hence, eriodictyol can interact more with the S2 pocket in NS3, as shown by its interaction with Asn152, a part of the protease active site. Similar to eriodictyol, eriodictyol-7-O-rutinoside interacts only with amino acid residues of NS3 protease. The amino acid residues of NS3 interacting with eriodictyol-7-O-rutinoside are TrpB:69, LysB:74, GlyB:148, LeuB:149, AsnB:152, AlaB:166, and AsnB:167. There are certain similarities between eriodictyol and eriodictyol-7-O-rutinoside in that they both bind to LysB:74 and LeuB:149 besides AsnB:152.

The interacting amino acid residues of NS3 protease with catechin gallate (Figure 3A) include LysB:74, LeuB:76, TrpB:83, TrpB:89, AsnB:152, AlaB:164, AlaB:166, and AsnB:167. The interacting amino acid residues of NS3 protease (Figure 3B) with luteolin-7-O-glucoside include LysB:74, LeuB:76, GlyB:87, GluB:88, ValB:147, LeuB:149, AlaB:164, IleB:165, and AsnB:167. Like eriodictyol and eriodictyol-7-O-rutinoside, catechin gallate also interacts with amino acid residue Asn152 in the S2 pocket of the NS3 protease. Although luteolin-7-O-glucoside does not interact with Asn152, like catechin gallate, it interacts with Lys74, Leu76, Ala164, and Asn167. Eriodictyol and eriodictyol-7-O-rutinoside also interact with Lys74, suggesting Lys74 as the major amino acid interacting with flavonoids.

The non-bonded interactions of various amino acid residues of NS3 protease with the four phytochemicals of *Lens culinaris*: eriodictyol, eriodictyol-7-O-rutinoside, catechin gallate, and luteolin-7-O-glucoside, including the nature of the formed bonds, are given in Table 2. In summary, the selected phytochemicals from *Lens culinaris* mainly employ hydrogen bonding and hydrophobic interactions in their predicted docked poses to NS3 protease (Table 2). Furthermore, the multiple hydrogen bonding and hydrophobic interactions confirm the strong binding affinity of these four phytochemicals with NS3 protease (Table 1) (Panigrahi and Desiraju, 2007).

As a number of the phytochemicals of *Lens culinaris* appeared to have good docking scores indicative of good affinity, we sought out to predict whether or not those molecules possess acceptable pharmacokinetic profiles as drugs or leads for developing drugs. For this purpose, the SwissADME server (http://www.swissadme.ch/) was used, and some of the salient features predicted this way are given in Table 3.

Out of the four compounds with high binding affinities (Figure 4), only eriodictyol-7-O-rutinoside showed 3 violations indicating that the gut absorption of this compound will be poor. In contrast, the other three compounds eriodictyol, catechin gallate, and luteolin-7-O-glucoside had 0, 1, and 2 violations, respectively, suggesting them as good drug candidates against DENV serotype 2 (Table 3).

Various flavonoid compounds have been reported previously as effective inhibitors for different DENV serotypes. Naringin, catechin, fisetin, naringenin, delphinidin, and epigallocatechin gallate have been shown to be inhibitory against DENV-2 in Vero cells (Loaiza-Cano et al., 2020). Other flavonoids, including amentoflavone, quercetin-3-O-β-D-glucopyranoside, avicularin, reynoutrin, silymarin, and scutellarin, were reported as potential lead candidates to stop viral replication of DENV serotypes

Table 2: Non-bonded interactions of selected phytochemicals of *Lens culinaris* with NS3 protease.

Phytochemicals	**Residues**	**Distance**	**Category**	**Type**
Eriodictyol	LEU149	2.25	Hydrogen Bond	CH
	ASN152	2.35	Hydrogen Bond	CH
	GLY87	2.51	Hydrogen Bond	CH
	VAL146	2.09	Hydrogen Bond	CH
	LEU85	2.71	Hydrogen Bond	CH
	TRP83	5.15	Hydrophobic	Pi-Pi T-shaped
	LYS74	4.10	Hydrophobic	Pi-Alkyl
	LEU76	4.92	Hydrophobic	Pi-Alkyl
Eriodictyol-7-O-rutinoside	TRP69	2.53	Hydrogen Bond	CH
	LEU149	2.85	Hydrogen Bond	CH
	ASN152	2.83	Hydrogen Bond	CH
	ASN167	2.03	Hydrogen Bond	CH
	LYS74	3.28	Hydrogen Bond	CH
	GLY148	3.78	Hydrogen Bond	C
	TRP69	5.36	Hydrophobic	Pi-Pi Stacked
	TRP69	4.87	Hydrophobic	Pi-Pi Stacked
	ALA166	3.74	Hydrophobic	Alkyl
Catechin gallate	ASN167	2.17	Hydrogen Bond	CH
	TRP89	2.32	Hydrogen Bond	CH
	ALA164	2.65	Hydrogen Bond	CH
	LYS74	4.38	Electrostatic	Pi-Cation
	LEU76	3.56	Hydrophobic	Pi-Sigma
	ALA166	3.70	Hydrophobic	Pi-Sigma
	TRP83	4.21	Hydrophobic	Pi-Pi Stacked
	TRP83	5.70	Hydrophobic	Pi-Pi Stacked
	TRP83	5.00	Hydrophobic	Pi-Alkyl
	LYS74	4.64	Hydrophobic	Pi-Alkyl
Luteolin-7-O-glucoside	LEU149	2.17	Hydrogen Bond	CH
	LEU149	2.52	Hydrogen Bond	CH
	VAL147	2.19	Hydrogen Bond	CH
	GLY87	2.29	Hydrogen Bond	CH
	GLU88	3.57	Hydrogen Bond	C
	LEU76	3.50	Hydrophobic	Pi-Sigma
	LEU76	3.87	Hydrophobic	Pi-Sigma
	ALA164	3.88	Hydrophobic	Pi-Sigma
	ILE165;ALA166	5.32	Hydrophobic	Amide-Pi Stacked
	ILE165;ALA166	4.34	Hydrophobic	Amide-Pi Stacked
	LYS74	5.06	Hydrophobic	Pi-Alkyl
	LYS74	4.82	Hydrophobic	Pi-Alkyl

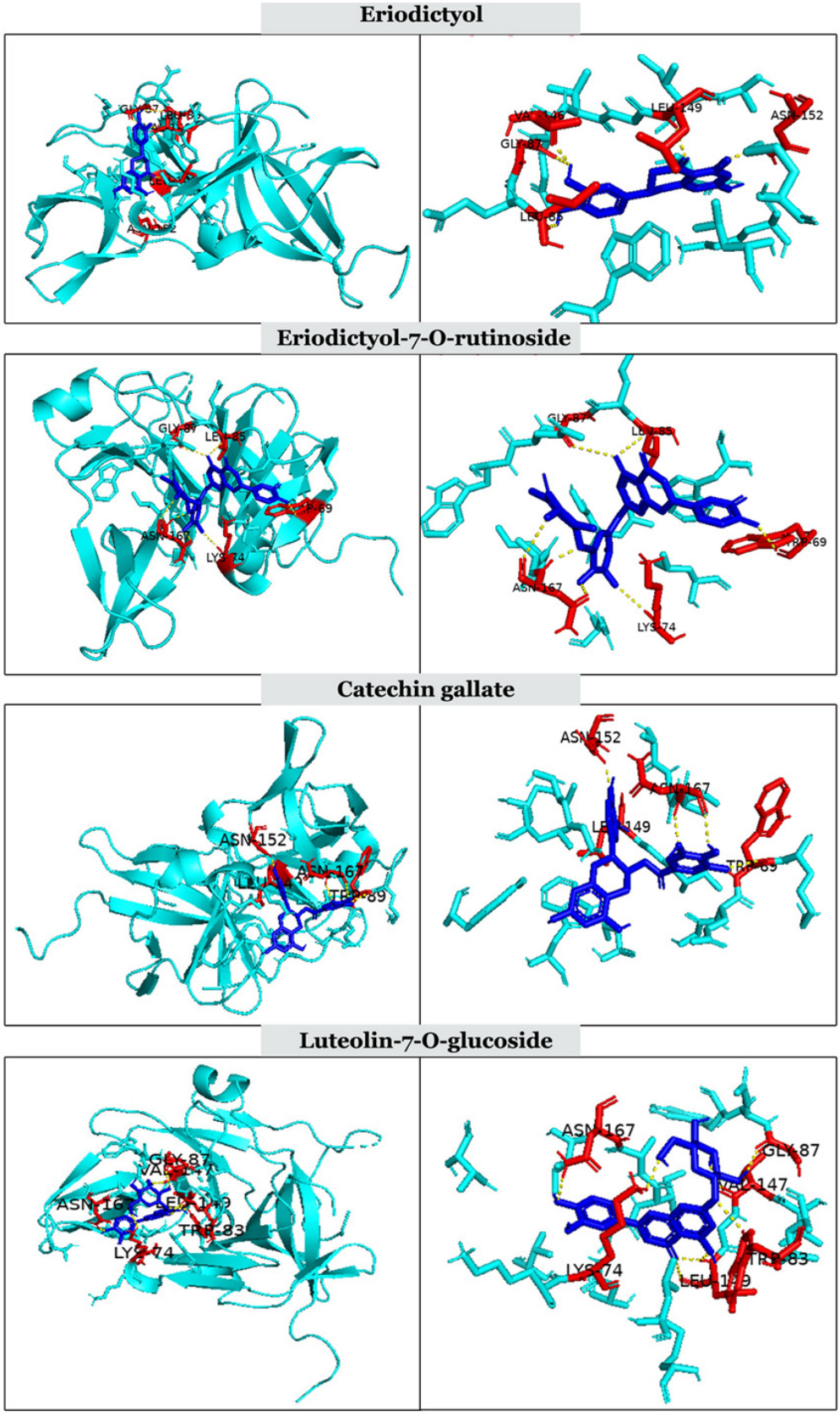

Figure 4: The PyMol depiction of Eriodictyol, Eriodictyol-7-*O*-rutinoside, Catechin gallate, and Luteolin-7-*O*-glucoside interactions with NS3 protease; an enlarged view (left) of the key amino acids (red) interacting with the ligand (blue) is shown as stick representation. These residues and the nature of interactions are also listed in Table 2.

Table 3: Physico-chemical properties of selected compounds of *Lens culinaris* predicted by SwissADME.

Phytochemical	Molecular weight	No of H-Bond acceptors	No of H-Bond donors	Log P	Molar refractivity	No of violation
(-)-Epigallocatechin	306.27	7	6	0.98	76.36	1
(+)-Catechin-3-O-glucose	452.41	11	8	-0.19	106.72	2
Catechin	290.27	6	5	1.47	74.33	0
Catechin-7-O-glucoside	452.41	11	8	1.55	106.46	2
Catechin gallate	442.37	10	7	1.31	110.04	1
Epicatechin	290.27	6	5	1.47	74.33	0
Epicatechin gallate	442.37	10	7	1.31	110.04	1
Quercetin-3-O-glucoside	464.38	12	8	2.11	11.16	2
Quercetin	302.24	7	5	1.63	78.03	0
Querctin-3-O-galactoside	464.38	12	8	2.11	110.16	2
Quercetin-3-O-xyloside	434.35	11	7	1.61	104.19	2
Kaempferol-4'-O-glucoside	448.38	11	7	2.04	108.13	2
Kaempferol-3-O-glucoside	448.38	11	7	0.53	108.13	2
Kaempferol-3-O-rutinoside	594.52	15	9	2.79	139.36	3
Myricetin-3-O-rhamnoside	464.38	12	8	0.92	111.02	2
4''''-Acetylsagittatin A	752.67	19	9	2.96	174.08	3
Procyanidin	594.52	13	10	1.70	147.52	3
Prodelphinidin	610.52	14	12	1.76	150.76	3
Eriodictyol	288.25	6	4	1.62	73.59	0
Eriodictyol-7-O-rutinoside	596.53	15	9	1.95	136.94	3
Naringenin	272.25	5	3	1.75	71.57	0
Apigenin	270.24	5	3	1.89	73.99	0
Luteolin	286.24	6	4	1.86	76.01	0
Luteolin-4'-O-glucoside	448.38	11	7	2.02	108.13	2
Luteolin-3',7-diglucoside	610.52	16	10	2.07	140.26	3
Luteolin-7-O-glucoside	448.38	11	7	1.83	108.13	2
5,7-Dimethoxyflavone	282.29	4	0	2.95	80.90	0
Malvidin-3-O-galactoside	528.89	12	7	-3.24	125.11	3
Resveratrol	228.24	3	3	1.71	67.88	0
Trans-Resveratrol 3-O-b-glucoside	390.38	8	6	1.75	100.00	1
Lutein	568.87	2	2	7.15	186.76	3
Stigmasterol	412.69	1	1	4.96	132.75	0
Campesterol	400.68	1	1	4.92	128.42	0
α-Sitosterol	414.71	1	1	4.79	133.23	0

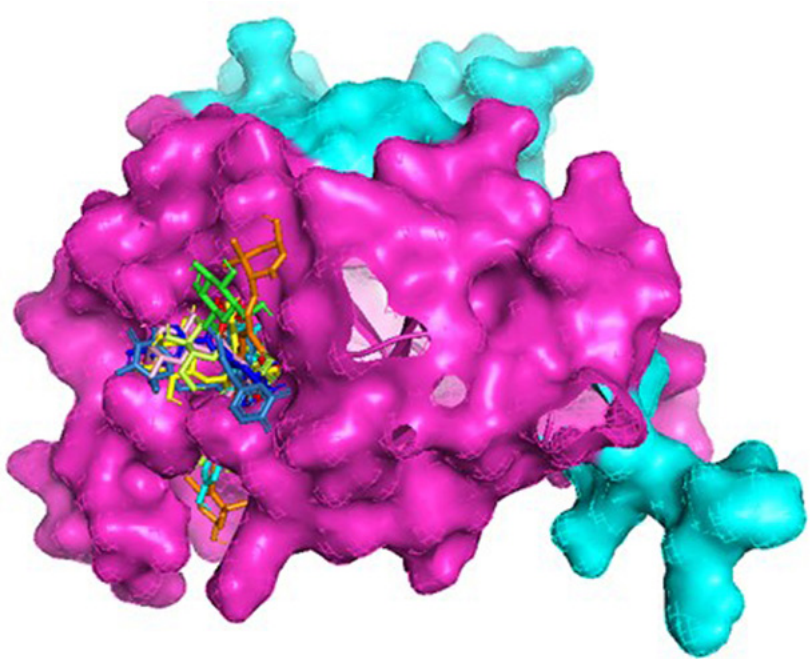

Figure 5: Surface diagram of binding of selected phytochemicals to NS2B-NS3 protease complex. The NS3 protease (magenta colour surface) and the NS2B activator part (blue colour surface). The tested phytochemicals do not bind to the protease-activator interface; instead, they bind to the protease. Blue-Catechin gallate; Yellow- Querctin-3-O-galactoside; Green-Kaempferol-4'-O-glucoside; Cyan- Trans-Resveratrol 3-O-b-glucoside; Orange-Lutein; Red-Eriodictyol; Sky Blue-Eriodictyol-7-O-rutinoside; Lemon-Luteolin-7-O-glucoside; and Pink-Malvidin-3-O-galactoside.

1 and 4 (Jayadevappa et al., 2020). In a sense, the present study is an improvement over previous studies in that 43 compounds coming from both flavonoid and non-flavonoid groups were evaluated for DENV-2 inhibitory potential. The limitation of this study is that molecular docking and other analyses were performed only on DENV serotype 2 NS2B-NS3 protease-activator complex. However, the tested phytochemicals with the least binding energies bind only to NS3 protease (Figure 5). Additionally, proteases the main DENV serotypes 1-4 are highly conserved between (Aguilera-Pesantes et al., 2017), suggesting that these phytochemicals will possibly bind with similar affinities to NS3 protease of the other DENV serotypes. However, this limitation needs to be addressed in future studies.

Conclusions

A total of forty-three phytochemicals from *Lens culinaris* have been evaluated through *in-silico* studies (molecular docking) for their binding potential to the active site of NS2B-NS3 protease of DENV-2 serotype. The phytochemicals belonged to both flavonoid and non-flavonoid groups like hydroxybenzoic and hydroxycinnamic acids, carotenoids, and phytosterols. Out of the forty-three phytochemicals, eight compounds (all non-flavonoids) showed binding energies higher than -7.0 kcal/mol, suggesting that they would be suitable inhibitors of NS2B-NS3 protease. The 2D and PyMol depictions of several compounds of high binding affinities showed that interactions of these compounds are mainly with the NS3 protease but not the NS2B activator protein. The violations of Lipinski's Ro5 analysis of four compounds with the highest binding affinities revealed that three compounds, i.e. eriodictyol, catechin gallate, and luteolin-7-O-glucoside have 0, 1, and 2 violations, respectively, suggesting them as promising therapeutics against DENV serotype 2 and possibly other DENV serotypes, however, this will have to be determined in similar studies.

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References

Ab-Fatah, M., Subenthiran, S., Abdul-Rahman, P.S.A., Saat, Z., Thayan, R., 2015. Dengue serotype surveillance among patients admitted for dengue in two major hospitals in Selangor, Malaysia, 2010-2011. Tropical Biomedicine 32, 187–191. URL: https://www.ncbi.nlm.nih.gov/pubmed/25801270.

Adebote, D.A., Kogi, E., Oniye, S.J., Akoje, F., 2011. Epidemiological significance of the breeding of mosquitoes in discarded automobile tyres in Zaria, Northern Nigeria. The Journal of Communicable Diseases 43, 183–192. URL: https://www.ncbi.nlm.nih.gov/pubmed/23781631.

Aguilera-Pesantes, D., Robayo, L.E., Méndez, P.E., Mollocana, D., Marrero-Ponce, Y., Torres, F.J., Méndez, M.A., 2017.

Discovering key residues of dengue virus NS2b-NS3-protease: New binding sites for antiviral inhibitors design. Biochemical and Biophysical Research Communications 492, 631–642. 10.1016/j.bbrc.2017.03.107.

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., Brownstein, J.S., Hoen, A.G., Sankoh, O., Myers, M.F., George, D.B., Jaenisch, T., Wint, G.R.W., Simmons, C.P., Scott, T.W., Farrar, J.J., Hay, S.I., 2013. The global distribution and burden of dengue. Nature 496, 504–507. 10.1038/nature12060.

Brady, O.J., Gething, P.W., Bhatt, S., Messina, J.P., Brownstein, J.S., Hoen, A.G., Moyes, C.L., Farlow, A.W., Scott, T.W., Hay, S.I., 2012. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Neglected Tropical Diseases 6, e1760. 10.1371/journal.pntd.0001760.

Carrington, L.B., Simmons, C.P., 2014. Human to mosquito transmission of dengue viruses. Frontiers in Immunology 5, 290. 10.3389/fimmu.2014.00290.

Chairulfatah, A., Setiabudi, D., Agoes, R., Colebunders, R., 2003. Thrombocytopenia and platelet trasnfusions in dengue haemorrhagic fever and dengue shock syndrome. Dengue Bulletin 27, 138–143. URL: https://apps.who.int/iris/bitstream/handle/10665/163895/dbv27p138.pdf.

Cherrak, S.A., Merzouk, H., Mokhtari-Soulimane, N., 2020. Potential bioactive glycosylated flavonoids as SARS-CoV-2 main protease inhibitors: A molecular docking and simulation studies. Plos One 15, e0240653. 10.1371/journal.pone.0240653.

Constant, D.A., Mateo, R., Nagamine, C.M., Kirkegaard, K., 2018. Targeting intramolecular proteinase NS2B/3 cleavages for trans-dominant inhibition of dengue virus. Proceedings of the National Academy of Sciences of the United States of America 115, 10136–10141. 10.1073/pnas.1805195115.

Erbel, P., Schiering, N., D'Arcy, A., Renatus, M., Kroemer, M., Lim, S.P., Yin, Z., Keller, T.H., Vasudevan, S.G., Hommel, U., 2006. Structural basis for the activation of flaviviral NS3 proteases from dengue and West Nile virus. Nature Structural & Molecular Biology 13, 372–373. 10.1038/nsmb1073.

Falgout, B., Miller, R.H., Lai, C.J., 1993. Deletion analysis of dengue virus type 4 nonstructural protein NS2B: Identification of a domain required for NS2B-NS3 protease activity. Journal of Virology 67, 2034–2042. 10.1128/{JVI}.67.4.2034-2042.1993.

Fernandes, B.T., Segretti, C.F., Polli, M.C., Roberto, M.P., 2016. Analysis of the applicability and use of lipinski's rule for central nervous system drugs. Letters in Drug Design & Discovery 13, 999–1006. 10.2174/1570180813666160622092839.

Frimayanti, N., Zain, S.M., Lee, V.S., Wahab, H.A., Yusof, R., Abd Rahman, N., 2011. Fragment-based molecular design of new competitive dengue den2 ns2b/ns3 inhibitors from the components of fingerroot (*Boesenbergia rotunda*). In Silico Biology 11, 29–37. 10.3233/{ISB}-2012-0442.

Ganesan, K., Xu, B., 2017. Polyphenol-rich lentils and their health promoting effects. International Journal of Molecular Sciences 18. 10.3390/ijms18112390.

Giménez, B.G., Santos, M.S., Ferrarini, M., Fernandes, J.P.S., 2010. Evaluation of blockbuster drugs under the rule-of-five. Die Pharmazie 65, 148–152.

Hariono, M., Choi, S.B., Roslim, R.F., Nawi, M.S., Tan, M.L., Kamarulzaman, E.E., Mohamed, N., Yusof, R., Othman, S., Abd Rahman, N., Othman, R., Wahab, H.A., 2019. Thioguanine-based DENV-2 NS2B/NS3 protease inhibitors: Virtual screening, synthesis, biological evaluation and molecular modelling. Plos One 14, e0210869. 10.1371/journal.pone.0210869.

Hasan, A., Jahan, R., Jannat, K., Bondhon, T.A., Hossan, M.S., Mazumder, S., Fatima, A., Pereira, M.d.L., Wiart, C., Nissapatorn, V., Rahmatullah, M., 2020. Evaluation of *Lens culinaris* phytochemicals in binding to the 3C-like protease of SARS-CoV-2– A molecular docking approach. International Journal of Marine Science 72, 173–176. 10.25259/{IJMS_219_2020}.

Ihlenfeldt, W.D., 2018. PubChem. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. 10.1002/9783527806539.ch6e.

Isa, M.A., Mustapha, A., Qazi, S., Raza, K., Allamin, I.A., Ibrahim, M.M., Mohammed, M.M., 2020. In silico molecular docking and molecular dynamic simulation of potential inhibitors of 3C-like main proteinase (3CLpro) from severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) using selected African medicinal plants. Advances in Traditional Medicine 10.1007/s13596-020-00523-w.

Jayadevappa, M.K., Karkera, P.R., Siddappa, R.Y., Telkar, S., Karunakara, P., 2020. Investigation of plant flavonoids as potential dengue protease inhibitors. Journal of Herbmed Pharmacology 9, 366–373. 10.34172/jhp.2020.46.

Katzenmeier, G., 2004. Inhibition of the NS2B-NS3 protease-towards a causative therapy for dengue virus diseases. Dengue Bulletin 28, 58–67. URL: https://apps.who.int/iris/bitstream/handle/10665/163997/dbv28p58.pdf.

Kausar, M.A., Ali, A., Qiblawi, S., Shahid, S., Izhari, M.A., Saral, A., 2019. Molecular docking based design of dengue NS5 methyltransferase inhibitors. Bioinformation 15, 394–401. 10.6026/97320630015394.

Li, H., Clum, S., You, S., Ebner, K.E., Padmanabhan, R., 1999. The serine protease and RNA-stimulated nucleoside triphosphatase and RNA helicase functional domains of dengue virus type 2 NS3 converge within a region of 20 amino acids. Journal of Virology 73, 3108–3116. 10.1128/{JVI}.73.4.3108-3116.1999.

Li, J., Lim, S.P., Beer, D., Patel, V., Wen, D., Tumanut, C., Tully, D.C., Williams, J.A., Jiricek, J., Priestle, J.P., Harris, J.L., Vasudevan, S.G., 2005. Functional profiling of recombinant NS3 proteases from all four serotypes of dengue virus using tetrapeptide and octapeptide substrate libraries. The Journal of Biological Chemistry 280, 28766–28774. 10.1074/jbc.M500588200.

Loaiza-Cano, V., Monsalve-Escudero, L.M., Filho, C.d.S.M.B., Martinez-Gutierrez, M., Sousa, D.P.d., 2020. Antiviral role of phenolic compounds against dengue virus: A review. Biomolecules 11. 10.3390/biom11010011.

Mishra, B., Sharma, M., Pujhari, S.K., Ratho, R.K., Gopal, D.S., Kumar, C.N., Sarangi, G., Chayani, N., Varma, S.C., 2011. Utility of multiplex reverse transcriptase-polymerase chain reaction for diagnosis and serotypic characterization of dengue and chikungunya viruses in clinical samples. Diagnostic Microbiology and Infectious Disease 71, 118–125. 10.1016/j.diagmicrobio.2011.06.020.

Mustafa, M.S., Rasotgi, V., Jain, S., Gupta, V., 2015. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Medical Journal Armed Forces India 71, 67–70. 10.1016/j.mjafi.2014.09.011.

Nguyen, P.T.V., Yu, H., Keller, P.A., 2018. Molecular docking studies to explore potential binding pockets and inhibitors for chikungunya virus envelope glycoproteins. Interdisciplinary Sciences: Computational Life Sciences 10, 515–524. 10.1007/s12539-016-0209-0.

Owis, A.I., El-Hawary, M.S., El Amir, D., Aly, O.M., Abdelmohsen, U.R., Kamel, M.S., 2020. Molecular docking reveals the potential of *Salvadora persica* flavonoids to inhibit COVID-19 virus main protease. RSC Advances 10, 19570–19575. 10.1039/D0RA03582C.

Panigrahi, S.K., Desiraju, G.R., 2007. Strong and weak hydrogen bonds in the protein-ligand interface. Proteins 67, 128–141. 10.1002/prot.21253.

Paul, A., Vibhuti, A., Raj, S., 2016. Molecular docking NS4B of DENV 1-4 with known bioactive phyto-chemicals. Bioinformation 12, 140–148. 10.6026/97320630012140.

Rakib, A., Paul, A., Chy, M.N.U., Sami, S.A., Baral, S.K., Majumder, M., Tareq, A.M., Amin, M.N., Shahriar, A., Uddin, M.Z., Dutta, M., Tallei, T.E., Emran, T.B., Simal-Gandara, J., 2020. Biochemical and computational approach of selected phytocompounds from *Tinospora crispa* in the management of COVID-19. Molecules 25. 10.3390/molecules25173936.

Rothan, H.A., Han, H.C., Ramasamy, T.S., Othman, S., Rahman, N.A., Yusof, R., 2012. Inhibition of dengue NS2B-NS3 protease and viral replication in Vero cells by recombinant retrocyclin-1. BMC Infectious Diseases 12, 314. 10.1186/1471-2334-12-314.

Sarwar, M.W., Riaz, A., Dilshad, S.M.R., Al-Qahtani, A., Nawaz-Ul-Rehman, M.S., Mubin, M., 2018. Structure activity relationship (SAR) and quantitative structure activity relationship (QSAR) studies showed plant flavonoids as potential inhibitors of dengue NS2B-NS3 protease. BMC Structural Biology 18, 6. 10.1186/s12900-018-0084-5.

Stierand, K., Rarey, M., 2010. PoseView- molecular interaction patterns at a glance. Journal of Cheminformatics 2, P50. 10.1186/1758-2946-2-S1-P50.

Trott, O., Olson, A.J., 2010. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry 31, 455–461. 10.1002/jcc.21334.

World Health Organization, 2016. Dengue vaccine: WHO position paper – july 2016, 91, 349-364. . URL: http://www.who.int/wer.

World Health Organization, 2021. Dengue and severe dengue. URL: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue.

Yang, C.C., Hsieh, Y.C., Lee, S.J., Wu, S.H., Liao, C.L., Tsao, C.H., Chao, Y.S., Chern, J.H., Wu, C.P., Yueh, A., 2011. Novel dengue virus-specific NS2B/NS3 protease inhibitor, BP2109, discovered by a high-throughput screening assay. Antimicrobial Agents and Chemotherapy 55, 229–238. 10.1128/{AAC}.00855-10.

Yauch, L.E., Shresta, S., 2014. Dengue virus vaccine development. Advances in Virus Research 88, 315–372. 10.1016/B978-0-12-800098-4.00007-6.

Yin, Z., Patel, S.J., Wang, W.L., Chan, W.L., Ranga Rao, K.R., Wang, G., Ngew, X., Patel, V., Beer, D., Knox, J.E., Ma, N.L., Ehrhardt, C., Lim, S.P., Vasudevan, S.G., Keller, T.H., 2006. Peptide inhibitors of dengue virus NS3 protease. part 2: SAR study of tetrapeptide aldehyde inhibitors. Bioorganic & Medicinal Chemistry Letters 16, 40–43. 10.1016/j.bmcl.2005.09.049.