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FLAVONOID FROM CARICA PAPAYA, ANTIVIRAL COMPOUNDS THAT BLOCKS DENGUE VIRUS PROTEIN EXPRESSION

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ABSTRACT

Dengue hemorrhagic fever that is a globally health issue is caused by Dengue virus, a member of the *Flaviviridae* family. The viral NS2B-NS3 protease composite is essential for viral duplication and thus, it is recognized as a favorable anti-viral target. While there is no scientific proof to support the anti-dengue character of *Carica papaya* leaf extract, it is usually prescript for patients suffering from dengue fever. For this reason, we set out to investigate the antiviral characteristics of the compounds found in *Carica papaya* leaves against DENV-2. In this study, investigation on the anti-dengue activity of the extracts of the *Carica papaya* leaf using bioinformatics techniques was done. It is noteworthy that quercetin, a flavonoid, was shown to have the maximum bond energy to the NS2B-NS3 protease complex. This is demonstrated by the creation of amino acid residues with the six hydrogen at the receptor's obligatory site. According to findings of this study, *Carica papaya* flavonoids shows the important activity of anti-dengue.

Key words: ADMET, Dengue virus, NS2B-NS3 protease complex, Quercetin

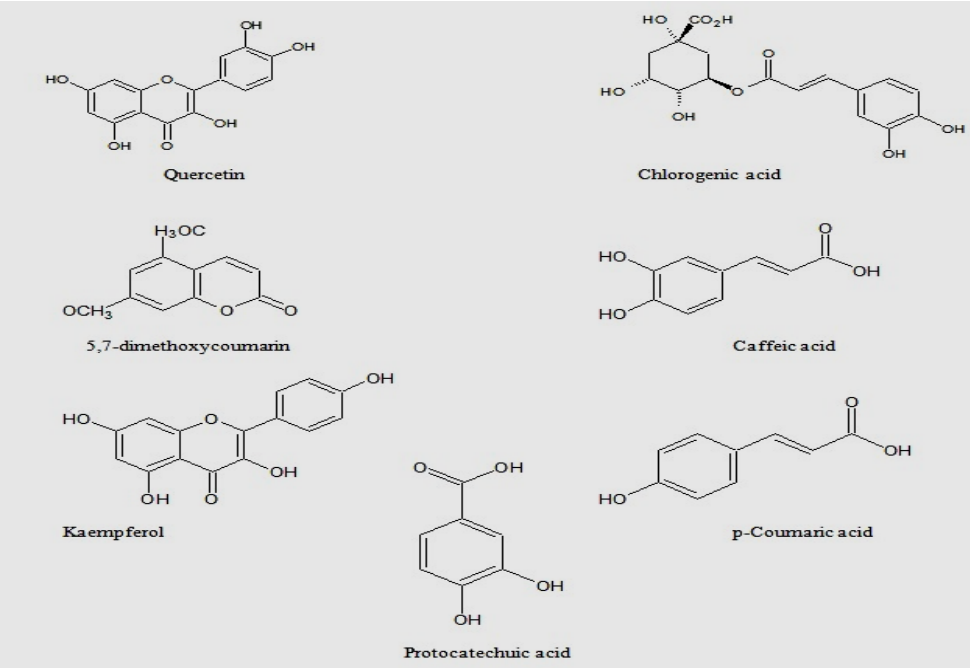
INTRODUCTION

Dengue fever is one of the significance public health distress globally. Recently, there is no availability of vaccine for inhibition and cure of DENV infection. DENV has a single stranded of positive-sense encapsulated RNA and genome consists of ten genes in which seven genes encode the seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) and three genes encode structural proteins (enveloped (E) glycoprotein, membrane-associated (M) protein and the nucleocapsid or core (C) protein).[1] DENV is belonged to flavivirus genus and a member of the Flaviviridae family. DENV is the cause of a broad range of clinical infections and disease, from quite mild, self-limiting illnesses with malaise, fever, and other unclear signs to more The compounds derived from flora plentiful resources for the making of innovative antiviral drugs. Several researches highlight the significance of phytochemical compounds in combating dengue virus.[3] *Carica papaya*, generally recognized as papaya is a herbaceous plant like a tree. It belongs to the Caricaceae, one of the plant families. The extracts of leaf of *Carica papaya* is approved as a boost for heart health and for the cure of diabetes, fever, gonorrhea, syphilis, pyrexia, inflammation, and for bandage tainted wounds.[4] Previous phytochemical analysis shows the existence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, phenolics, saponins, terpenoids, and phytosterols, inside the leave of *Carica papaya*. According to the previous study, the direction of *Carica papaya's* severe, lethal sickness with symptoms, cause the DHF and DSS. The dengue virus has four anti-genetically different but closely related serotypes: DENV1, DENV2, DENV3, and DENV4. A 65–70% sequence homology is revealed by those serotypes. The prime carriers for dengue transmission are the Aedes aegypti mosquito and some strains of Aedes albopictus. Of the four serotypes, DENV2 is one of the most prevalent.[2] NS2B and NS3, are the two main elements of viral serine protease, which play an necessary task in replication of virus, is requisite for the preparation of the polyprotein pioneer, that help the virus in its assembly mechanism. For making of anti-dengue medicines and drugs the presence of NS2B-NS3 complex is necessary otherwise drug or medicine is not efficient as anti dengue drugs.

aqueous leaf extracts shows the prospective anti-dengue features as indicate by the enhance the number of platelet from 0.53µL to 1.63µL, neutrophils from 46% to 78% and leukocytes count from 3.7x103µL to 7.7x103µL in blood of patients suffering with dengue fever. The GCMS analysis shows the seven phenol compounds (chemicals) kaempferol, quercetin, protocatechuic acid, caffeic acid, chlorogenic acid, 5,7-dimethoxycoumarin and p-coumaric acid are existing in the leaf of *Carica papaya* . Still, these seven phenolic chemicals are not investigated for their activities of anti-dengue. Therefore, we try to investigate this, and outcomes of the investigation propose that, for the viral components assembly, NS2B-NS3 protease complex is necessary and it activity will stop by the flavonoid quercetin. We regard, as this research

might be a best foundation of the produce of anti-dengue drugs and compounds from the leaf exacted of *Carica papaya*.

Figure 1: the seven different phenol compounds from the leave extract of



Cari *ca papaya*.

METHODOLOGY

3D STRUCTURE OF RECEPTOR

For the NS3's protease catalytic activity, the presence of the NS2B cofactor was crucial. From the the protein data bank (PDB ID: 2FOM), the 3D configuration of joining this NS3 protease and NS2B cofactor is and the 3D configuration of NS2B-NS3 complex of DENV-2 was retrieved from it. All H_2O molecules and previous ligand was detached and on the last phase, by using the software Discovery Studio 4.1, the target protein molecule was mixed with the hydrogen atoms.

COLLECTION OF ACTIVE COMPOUNDS

The literature reported by acted as a liberty to select the dataset for seven compounds from leaf extracts of *Carica papaya*. The 3D arrangement of seven compounds were repossessed from the ChemSpider (http://www.chemspider.com/) in MOL format, all the ligand molecules were transformed into PDB format by using the Discovery Studio 4.1 and then energy minimized. All the ligands were converted into PDBQT arrangement by using autodock_vina_1_1_2_win32. The configurationsof the ligands are shown in **(Figure 1).** Their molecular characteristics are shown in the **(Table 1)**.

MOLECULER DOCKING

Autodock_vina_1_1_2_win32, open-source software was used for Molecular docking simulation. At the beginning of the docking, the removing the unrelated substructure such as ligands and all water molecules was done by Discovery studio 4.1, to optimized the the protein receptor which is NS2B-NS3. Then the default setting like addition of hydrogen atoms used to fix the side chains of the protein

structure. Finally, the stable structure is saved as PDB file. Then this receptor PDB file was open in AutoDockTools-1.5.6. The receptor was chooses from the grid option and saved as a PDBQT file. Then the grid was made to create the docking target on the receptor molecule, their values of dimensions and center were noted. With the help of windows command prompt, the autodock_vina_1_1_2_win32 was ulitized to measure the interaction between the ligand and protein receptor, which evaluates binding affinities between the receptor and the ligand molecules individually. Then the ADMET profiling was done by the help of MedChem Designer.

DRUG SCORE

The online chemo informatics tool "Osiris Property Explorer" (http://www.organic-chemistry.org/prog/peo) used for Drug scan, which verify whether these seven compounds have satisfied the itself as a best drug applicant. Drug Score values are shown in the **(Table 8).**

RESULTS

MOLECULAR DOCKING

The autodock-vina- 1-1-2-win32 used for the docking of these seven ligand compounds onto the catalytic site of the protease serine enzyme NS2B-NS3**.** After docking, the best facade for each ligand compounds selection depended on the affinity (kcal/mol) with the protease. Quercetin is the compound with the highest binding affinity (kcal/mol) out of the seven compounds that were examined. Affinity gives several techniques for ligand-receptor interaction assessment.

Affinity is useful to decide configurations of diverse ligands bound to the same receptor or to rank various configurations of the same ligand. In order to have a deeper understanding of how ligands intermingle with the complex, binding energies of all ligands with multiple configurations are tabulated in **Table 2.** The quantity of hydrogen bonds development between the protein and the ligand finds the valuation of docking outcome. The result of molecular docking of the top constitutes attained is displayed in **Figure 2;** the ligand is displayed in stick replica while protein structure is displayed in surface model. In the **Figure 3** the major residues Ala 164, Asn 152, Asn 167, Gly 87, Leu 149, and Lys 74 are labeled and the six dot line are shown the six hydrogen bonds that are created between the compound quercetin serine protease is interacted with NS2B-NS3complex.

Table 1: Molecular characteristics of all these phenol compounds

S.No	Compounds	H-Bond Donor	ChemSpider ID	Molecular Weight [g/mol]	H-Bond Acceptor
1.	**Quercetin**	5	4444051	302.2	7
2.	**Chlorogenic Acid**	6	1405788	354.3	9
3.	**Citropten**	0	2473	206.1	4
4.	**Caffeic acid**	3	600426	180.1	4
5.	**Kaempferol**	4	4444395	286.2	6
6.	**p-Coumaric acid**	2	553148	164.1	3
7.	**Protocatechuic acid**	3	71	154.1	4

Table 2: Binding Affinities of all ligands with multiple configurations

Compound Name	1	2	3	4	5	6	7	8	9
Chlorogenic acid	-7.3	-7.3	-7.2	-7.2	-7.2	-6.9	-6.9	-6.8	-6.7
Citropten	-6.2	-6.1	-5.9	-5.7	-5.7	-5.6	-5.6	-5.6	-5.5
Kaempferol	-8.2	-7.9	-7.9	-7.7	-7.3	-7.3	-7.2	-7.0	-7.0
p-cumaric acid	-5.9	-5.8	-5.6	-5.4	-5.4	-5.4	-5.4	-5.4	-5.2
Protechuaic acid	-5.6	-5.4	-5.4	-5.3	-5.1	-5.1	-5.0	-5.0	-5.0
Quercitin	-8.4	-8.1	-8.1	-7.6	-7.6	-7.5	-7.4	-7.2	-7.1
t-cafeic acid	-6.0	-5.9	-5.8	-5.7	-5.6	-5.5	-5.4	-5.4	-5.3

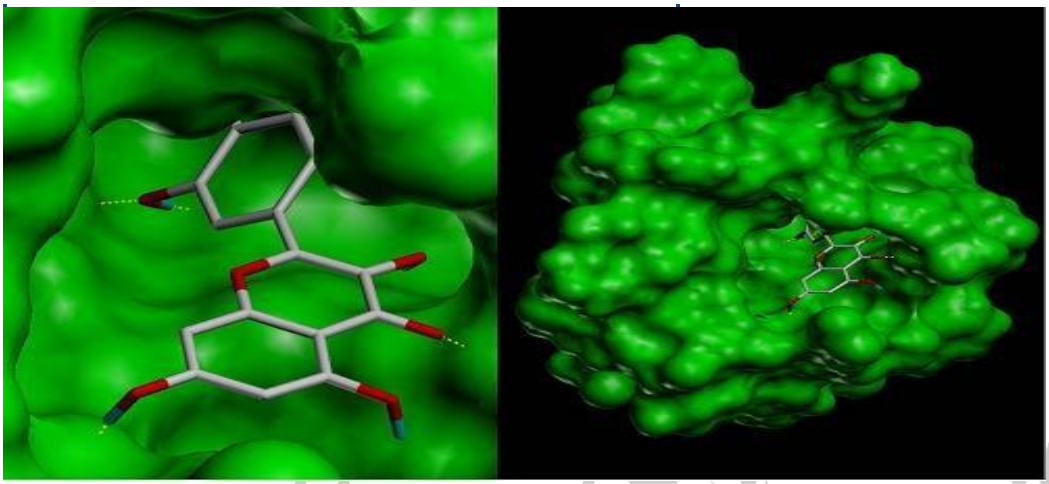


Figure 2: the NS2B-NS3 protease of the Dengue virus docked structure with the chemical quercetin

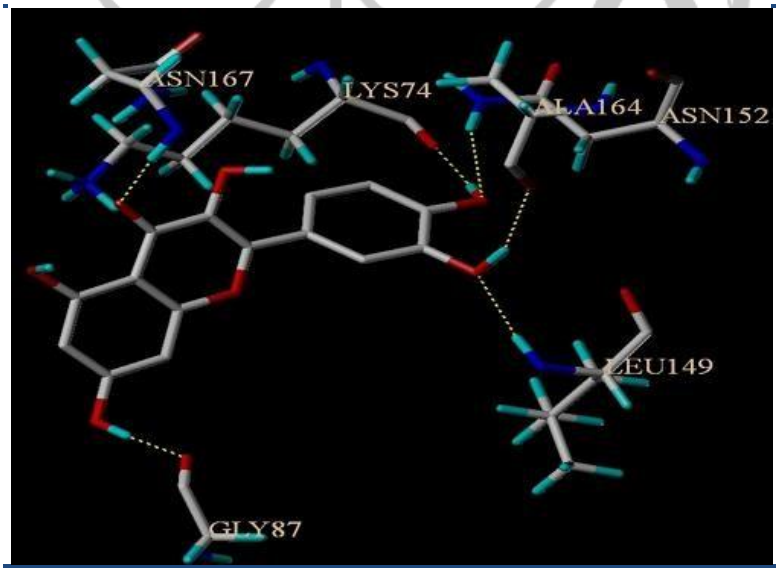


Figure 3: Interactions of hydrogen linkage between NS2B-NS3 protease complex and the chemical quercetin

ADMET PROPERTIES

The compounds' molecular characteristics, solubility and drugs likelihood are analyzed depend on "Lipinski's Rule of Five" using the tool Medchem Designer and result is shown in **Table 8**. The principle illustrates molecular characteristics essential for a pharmacokinetics of drug in the body of human being, as well as their distribution, absorption, excretion and metabolism. These seven chemicals are verified for the drug-likeliness and the outcomes are shown in **Table 3.** All of the seven chemicals, according to this study, passed by the filter without chlorogenic acid, which indicates one breach to the Lipinski's rules of five. Additionally, we used the admetSAR program (http://lmmd.ecust.edu.cn:8000/predict/) to evaluate the active compounds' ADMET profiles in silico. The admetSAR server forecasts the active compounds' ADMET-related characteristics for various model types, all of which generate hopeful outcomes. These outcomes are shown in **Table 3 to Table 7**.

Table 3: ADMET properties of compounds

Name of compound	Rule of 5	MlogP	M-NO	S+logP	HBDH	S+logD	T-PSA
Chlorogenic acid	1.000	-0.964	9.000	3.370	6.000	3.370	144.520
Citropten	0.000	0.635	4.000	1.183	1.000	1.779	58.530
Kaempferol	0.000	0.525	6.000	2.243	4.000	1.848	111.130
p-Cumeric acid	0.000	0.449	3.000	4.553	2.000	4.553	37.300
Protocatechuic acid	0.000	0.697	4.000	3.105	3.000	3.104	57.530
Quercitin	0.000	-0.235	7.000	1.958	5.000	1.529	131.360
t-caffeic acid	0.000	0.994	4.000	3.238	3.000	3.237	57.530

Table 4: ADMET: Absorption test predicted profile for active molecules (For Inhibitor + and For Non-Inhibitor -)

Models	BBB	$CaCO_2$ Permeability	HIA	P-glycoprotein Inhibitor	Renal Organic Cation Transporter
Quercetin	+	+	+	-	-
Caffeic acid	+	+	+	-	-
Chlorogenic acid	+	+	+	-	-
p-Coumaric acid	+	+	+	-	-
Kaempferol	+	+	+	-	-
Citropten	+	+	+	-	-
Protocatechuic acid	+	+	+	-	-

Table 5: Metabolism test for different strains of CYP450 substrate determined profile for active compound (For Structure + and for Non-Structure -)

Models	2C9	2D6	3A4
Quercetin	-	-	-
Caffeic acid	-	-	-
Chlorogenic acid	-	-	-
p-Coumaric acid	-	-	-
Kaempferol	-	-	-
Citropten	-	-	-
Protocatechuic acid	-	-	-

Table 6: Metabolism test for different strains of CYP450 Inhibitor determined the profile for active compound (For Inhibitor + and for Non-Inhibitor -)

Models	2C9	2C19	2D6	3A4
Quercetin	-	-	-	-
Caffeic acid	-	-	-	-
Chlorogenic acid	-	-	-	-
p-Coumaric acid	-	-	-	-
Kaempferol	-	-	-	-
Citropten	-	-	-	-
Protocatechuic acid	-	-	-	-

Table 7: Carcinogens and AMES Toxicity predicted profile for active compound

Models	Carcinogens	AMES Toxicity
Quercetin	NC	NT
Caffeic acid	NC	NT
Chlorogenic acid	NC	NT
p-Coumaric acid	NC	NT
Kaempferol	NC	NT
Citropten	NC	NT
Protocatechuic acid	NC	NT

Table 8: Drug Score of the active compounds

Compound Name	Solubility	Drug Likeness	Drug Score
Quercetin	-2.49	1.6	0.3
Chlorogenic Acid	-1.91	0.03	0.7
citropten	-1.78	-2.95	0.51
Caffeic Acid	-1.41	1.62	0.19
Kaempferol	-2.29	0.9	0.46
P-Coumaric Acid	-1.7	0.58	0.47
Protocatechuic Acid	-1.04	-0.12	0.43

DISCUSSION

Fever caused by Dengue virus has been come back as a critical being danger with enhancing the infectivity rates annually. The digits of pathogenically cases of serious dengue infections keeps on to raise widespread in regions of South and Central America, Southeast Asia, and other subtropical areas.[5] For the treatment of epidemic virus, it is crucial to generate effective anti-dengue chemicals. In this research, we inspected the chemicals found in Carica papaya leaf extracts in relation to the DENV2 virus's NS2B-NS3 serine protease. NH2-C-prME-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH is the sequence in which the single stranded RNA DENV2 codes for a single polyprotein precursor. RNA of DENV2 has a type I cap structure at its 5' end.[6] It is familiar that the N-terminal protease domain (NS3pro) of DENV-NS3 is a multi-functional protein. The two viral proteins called NS3 and NS2B, which are attached to each other and create a hetero complex, make up the viral protease. Viral replication needs the complex that the NS3 protease's N-terminal site makes with the NS2B cofactor. The concentration of virion offspring produced would decrease if discharge of the particles of the virus were prohibited. This would obstruct the virus's genomic replication. Based on our investigation, quercetin was determined to be one of the best inhibitors because of its superior stability and ligand-enzyme interactions. P-coumaric acid display the maximum binding affinity for hydrogen bonds; though its ligand-receptor atom pair interaction values are confirmed to be low. In contrast to quercetin, chlorogenic acid, kaempferol, and citropten or 5, 7-dimethoxycoumarin forms five hydrogen bonds with the NS2B-NS3 protease active site residues. But, their binding affinity is least. Regarding hydrogen bond interactions, caffeine had the fewest—four—among the seven chemicals examined. One of the major barriers in the drugs development process is evaluating the lead compounds' absorption, distribution, metabolism, and excretion (ADME) properties.[7] The main two reasons toxicity and low pharmacokinetic qualities, which are the causes for most compounds to fail in the drug development process**.** The detection of active lead compounds in the early stages of drug discovery is made possible by the development of rapid and high throughput ADMET profiling tests. The efficient lead compounds are developed during the drug design process, credit to ADME profiling tests during the early stages of the discovery process. According to ADME profiling test, except chlorogenic acid all of the active chemicals, have no

negative effects on absorption. The ability of the active compounds to behave as drugs is strongly supported by the optimistic outcome of ADMET-linked properties for various models. The group of isozymes called as cytochrome P450 (CYP) is involved in the metabolism of fatty acids, steroids, medicines, carcinogens, bile acids and drugs. Almost 57 CYP are encoded in the human genome, 15 of which are involved in the pharmaceuticals and drugs and metabolism of xenobiotics. A CYP enzyme is the base of Phase I drug metabolism on participation approximately 72 to 75 % of cases. [5] When it comes to metabolism, a various kinds of CYP inhibitor-substrate complex models are estimated, the outcome illustrates that those substances are neither CYP enzyme substrates nor inhibitors. It is discovered that none of the chemicals or compounds are toxic and hazardous. Result of this study expose that the quercetin may have shown inhibitory effect against NS2B-NS3 serine protease, out of the seven substances examined. Further, it is strongly suggested toxicity risk assessment and ADME powerfully propose that quercetin has powerfully antiviral efficacy against the virus of DENV2. According to data of the study, we propose that, the flavonoid quercetin in Carica papaya leaf extract may slow down or restrain the DENV2 virus's ability to assemble itself and ready for infection. Based on our findings, quercetin comes out to be the most promising option for creating effective anti-dengue chemicals. Overall, we draw the conclusion that quercetin, a flavonoid, virtues more research and may one day be produced into an effective anti-dengue agent.

Abbreviations:

ADME	Absorption, Distribution, Metabolism & Excretion
BBB	Blood Brain Barrier
CYP	Cytochrome P450
DENV	Dengue virus
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
GCMS	Gas chromatography
HIA	Human Intestinal Absorption
MS	Mass spectrometry
NI	Non-inhibitor
NC	Non-carcinogen
NS	Non-structural
NT	Non-toxic

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