doi.org/10.36721/PJPS.2020.33.1.SUP.355-360.1

Exploration of *Carica papaya* bioactive compounds as potential inhibitors of dengue NS2B, NS3 and NS5 protease

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Abstract: Current outbreak of dengue has shown serious health concerns in Pakistan. The present study reports the anti-dengue potential of *Carica papaya* natural compounds. The leaves of *C. papaya* have previously shown promising results in cure of Dengue fever. The aim of this project is to find specific bioactive compounds by computational screening and biological activities of *C. papaya* against serine NS2B, NS3 and NS5 proteases of dengue virus. Docking study resulted in the screening of nine bioactive compounds having highest docking scores. However, three compounds namely epigallocatchin, catechin and protocatechuric acid had the strongest binding affinity with the active residues i.e., Ser135, His51 and Asp75 of dengue virus serine proteases. Results also indicated that the extract of *C. papaya* was a strong antimicrobial and antioxidant agent. It is concluded that the *C. papaya* compounds can be commercially applied for medical formulations against dengue virus.

Keywords: Phytochemicals, antimicrobial, antioxidant, non-structural proteins, *C. papaya.*

INTRODUCTION

Dengue fever is the leading cause of health problems across the globe and especially in underdeveloped countries like Pakistan (Ahmad *et al*., 2011, Singh *et al.,* 2017). It is a viral disease caused by dengue virus (DENV) which is transmitted through a special type of mosquito. The DENV is single stranded RNA virus of *Flaviviridae* family and *Flavivirus* genus (Rodenhuis-Zybert *et al.,* 2010). There are four known serotypes of virus responsible for the disease. Therefore, it is impossible to design a vaccine which is simultaneously effective against all these stereotypes. Dengue hemorrhagic fever results in major loss of platelets. Medicinal plants contain large number of naturally occurring bioactive compounds such as saponins, polyphenolics, terpenoids, flavonoids, peptides, thiophenes, coumanrins, ploynes, alkaloids, organosulfur compounds and limonoids (Debnath *et al*., 2003). These bioactive compounds are more effective inhibiters of NS2B/NS3 of dengue virus RNA with negligible side effects (Takshak *et al*., 2018).

C. papaya (Caricaceae) is found in tropical and sub-tropical regions around the world. *C. papaya,* a well-known plant used in folk medicines, was found to be the best anti-dengue agent (Ahmad *et al*., 2011, Srivastava and Singh, 2016). It has some immunity enhancing elements which are useful against the dengue virus (Ahmad *et al*., 2011). Various phytochemicals including alkaloids, flavonoids, phenols and antioxidants are present in sufficient amount in papaya plant. The papain extracts of the leaves are useful in the treatment of digestive disorders. The extracts from fruits and seeds have bactericidal properties. The fruit juice and leaf extract have been demonstrated to exhibit a wide variety of properties including anticancer, antioxidative, anti-inflammatory, anti-bacterial, nephroprotective, hepatoprotective, hypoglycemic, hypolipidemic and anti-sickling effects in sickle cell disease (Bozin *et al*., 2006). The ripe fruit has been used against ringworm, whereas the green fruit has been used to lower blood pressure, as an aphrodisiac and to induce abortion. The leaf extract has also been shown to have larvicidal properties against the *Aedes aegypti* mosquito, the vector of the DENV (Sarala *et al.,* 2014).

The papaya leaves are packed with certain useful enzymes like papain and chymopapin. Papain, also known as papaya proteinase I, is a cysteine protease present in papaya (Azarkan *et al*., 2006). The possible mode of action of such enzymes show that these are useful for enhancing the blood platelet count and blood clotting factor in human. This activity of papaya plant is due to papase or papin enzyme whose concentration is low in papaya plant. Moreover, this plant is not available throughout the world and is cultivated in specific areas of subcontinent, therefore, it was necessary to hyperproduce that enzyme and collect the reasonable amount for the treatment of dengue. The purpose of this study was to screen the bioactive compounds of papaya for their activity as inhibitors of dengue proteins NS2B, NS3 and NS5.

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MATERIALS AND METHODS

The study was approved by ethical review committee of Government College University, Faisalabad. Ref. No. GCUF/ERC/4187 dated 27-04-2016.

Molecular docking

Molecular docking analysis was carried out in a previous study to screen about 900 phytochemicals in *C. papaya* and to identify those phytochemicals which were inhibitors of dengue virus NS2B, NS3 and NS5 proteases. The 3D structure of dengue virus NS2B, NS3 and NS5 proteases (proteins) were retrieved from the PDB (Protein Data Bank). The bioactive compounds of the plant were retrieved from MAPS, MPD3 and PubChem databases (Ashfaq *et al*., 2013; Velmurugan *et al*., 2014). MOE was used to scan the inhibitors of NS2B, NS3 and NS5 protease. Later on, residues (Asp75, Ser135, His51) interacting with the above-mentioned proteases were recognised and selected by MOE. The structure of phytochemicals was appointed according to higher and lower values of S-score and RMSD respectively. The phytochemicals having such values were only selected for ADMET profiling. Finally, nine bioactive compounds were found to be best scored and analyzed through the Lipinski's rule of five (Lipinski *et al*., 2004). The Swiss ADME software was used to evaluate the drug like characteristics of selected candidates.

Sample collection and extract preparation

C. papaya plant material (leaves, fruit, stem, root and seeds) were collected from District Bahawalnagar from April-July 2019 and identified by the Taxanomist. The plants were washed with water, sliced into small pieces and spread like thin layers in trays and air dried. The dried parts were ground to coarse powder with the help of blender to facilitate the easy penetration of solvent into the cells to extract the constituents.

The fine powder of leaves, fruit, stem, root and seeds of papaya were extracted in water, hexane, buffer and methanol. The extracts were filtered using muslin fabric and filter paper. Then it was kept in rotary evaporator for complete evaporation of the solvent and a gummy extract was obtained which was preserved in refrigerator.

Fourier transform infrared (FTIR) spectrophotometery) of papaya

FTIR was performed at Central Hi-Tech Lab of Government College University, Faisalabad, using FTIR spectrometer, spectrum 2 (Perkin Elmer, USA). Ten mg of fresh extract dust was encapsulated in 100 mg KBr pellet. Scanning spectrum was preserved from 400 to 4000 cm and a precision of 4 cm. The results of this study produced the FTIR spectrum picture of *C. papaya* leaves, grains, roses and fruit milk individually.

Antimicrobial activity

Strains of *B. subtilitis, B. megaterium, B. cerus, B. haemolytic streptococcus,* and *C. albicans* were obtained from the Microbiology Department in University of Agriculture, Faisalabad. Lauria and Bertani (LB) media were used for the preservation of bacterial strains while Eggins and Pugh (E and P) medium was used to preserve fungal strains. Standard microbiological protocols were followed in handling the microbial strains. The antimicrobial activity of *C. papaya* extract was determined by disk agar diffusion method. The activity was performed according to Ates *et al*. (2003). Standard antibiotic control disc having 30 µg of kanamycin was used.

DPPH scavenging activity

The DPPH radical scavenging exercise assay was performed with small changes in the technique of Yen and Chen (1995). One mL of 0.004% 2, 21-diphenyl-1-picrylhydazyl (DPPH) in methanol water (freshly prepared) was mixed to 3 mL of seed, fruit, leaves and root extracts of *C. papaya*. The sample was incubated at 25 ^{o}C for 1 hour and the absorbance was measured at 515 nm using a spectrophotometer. The DPPH radical samples ratio inhibition was calculated as follows.

DPPH Inhibition (%) = Absorbance of Blank – Absorbance of Sample/ Absorbance of Blank ×100

STATISTICAL ANALYSIS

The data was calculated using statistical tools SPSS 19 for means. Linear regression equation was used to calculate activities through standard curve. Linear model function (one way ANOVA) was used to find significant difference in zones of inhibition for various microorganisms.

RESULTS

Molecular docking

The present study was designed to explore potential of *C. Papaya* by docking their active compounds with non-structural proteins NS2B, NS3 and NS5 of dengue. Some previous studies used different approaches to find potent inhibitors of dengue virus NS2B and NS3 proteases by using several synthetic, natural and cyclopeptide molecules (Idrees and Ashfaq, 2012). Similarly, Idrees and Ashfaq (2014) designed different molecules and docked them against dengue virus NS2B, NS3 protease.

The library of almost 900 bioactive phytochemicals of *C. papaya* was docked against the NS2B, NS3 and NS5 protease and ranked according to S-score, RMSD value and binding affinity with the proteases. Nine compounds (Protocatechuric acid, Genistein, Epigallocatchin, Baicalein, 1-Hydroxy-2-propanone, Catechin, Fisetin, 2-

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

S. No	Compound name	Docking Score	RMSD value	Residues
A	1-Hydroxy-2-propanone	-5.6786	1.0895	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
B	2-methyl-propanoic acid	-6.8851	1.5143	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
C	Baicalein	-7.2802	2.1897	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
D	2-Methyl-butanoic acid	-6.4896	2.1092	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
E	Fisetin	-7.5123	3.2903	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
F	Epigallocatchin	-13.2911	3.5421	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
G	Genistein	-7.4233	2.4439	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
H	Catechin	-9.0122	3.4011	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151
I	Protocatechuric acid	-7.5592	1.9586	His51,Asp75,Ser135, Val72,Lys73,Tyr135, Gly151

Table 2: ADMET profiling results of top nine *C. papaya* bioactive compounds

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

Table 3: DPPH scavenging activity of *C. papaya*

C. Papaya	% Inhibition
Fruit	86.5
Leaves	82.2
Seed	65.9
Root	64.7

Table 4: Antimicrobial activity of *C. papaya*

Microorganisms			Zone of inhibition (mm)		
		Leaf	Fruit	Seed	Kanamycin
Gram Positive Bacteria	*B. subtilitis*	25^{b}	27^{b}	18^{c}	32^{a}
	B. megaterium	27^{b}	28^{b}	15^{c}	33^{a}
	B. cerus	28^{b}	25^{b}	19^{c}	33^{a}
	B. haemolytic streptococcus	22^{b}	24^{b}	16^{c}	32^{a}
Fungi	*C. albicans*	23^{bc}	24^{b}	18^{c}	32^{a}
	A. niger	26^{b}	22^{c}	19^{cd}	33^{a}
	R. solani	25^{b}	27^{b}	18^{c}	33^{a}

Inhibition zone with different letters differ significantly as compared by LSD at alpha level 5%.

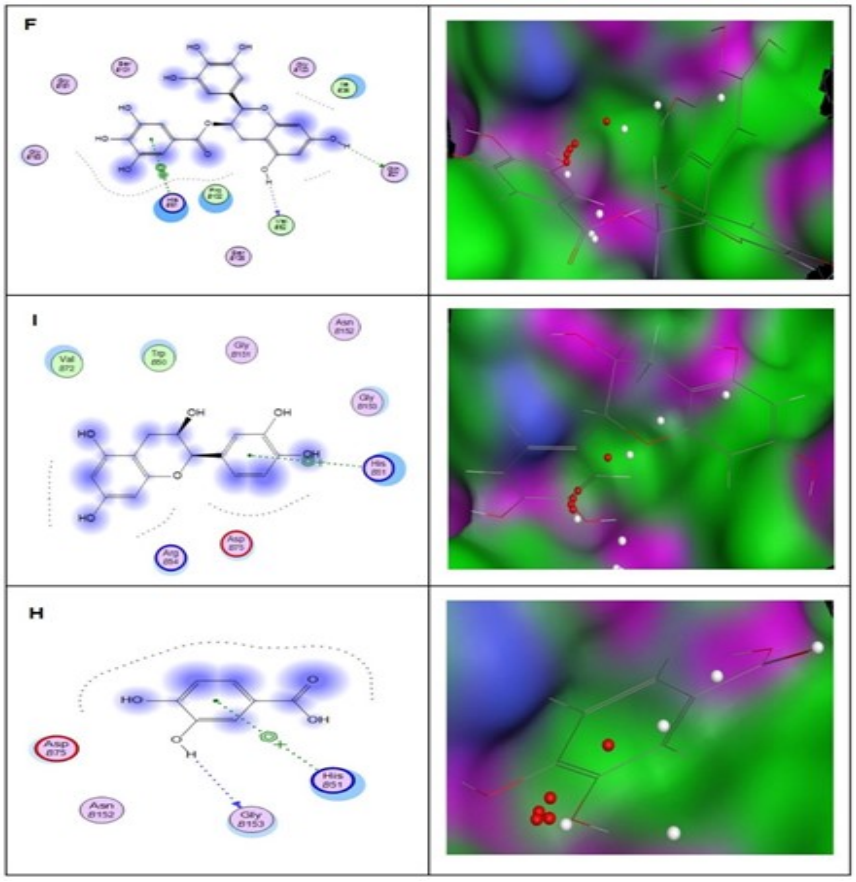

Fig. 1: 2D and 3D interactions of bioactive compounds (ligands) from *C. papaya* with dengue NS2B, NS3 and NS5 serine protease.

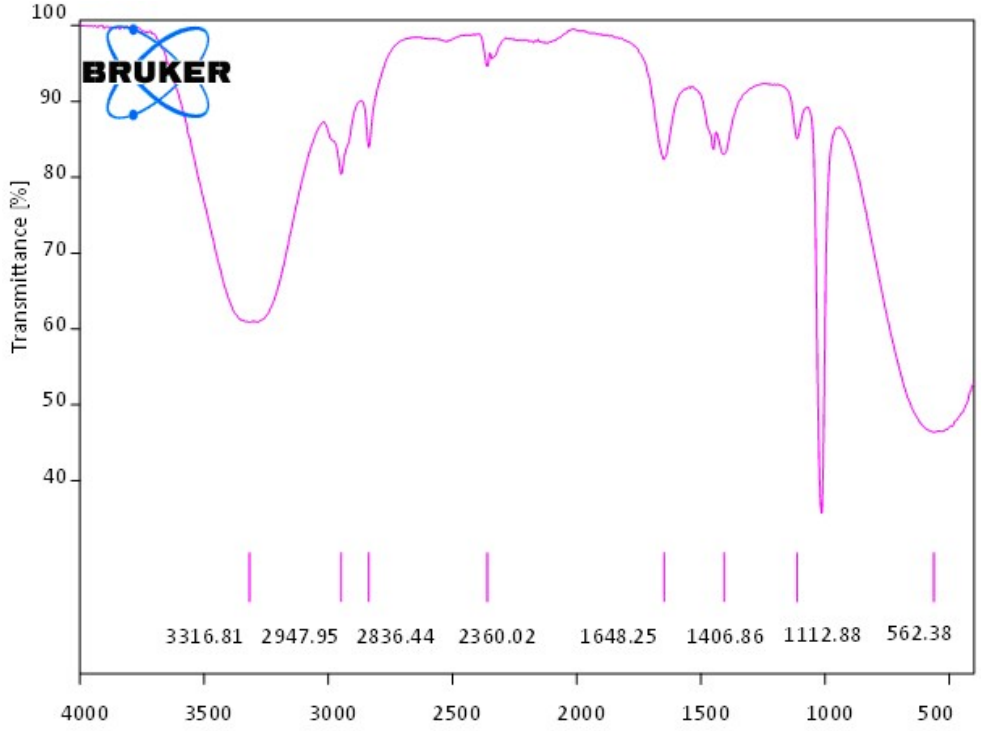

Fig. 2: FTIR analysis of methanol (ME) extract of *C. papaya* leaves.

Methyl-butanoic acid, 2-Methyl-propanoic acid) were at the top and selected. The docking scores of top ranked nine compounds -5.6786, -6.8851, -7.2802, -6.4896, -7.5123, -13.2911, -7.4233, -9.0122, -7.5592 and RMSD values were 1.0895, 1.5143, 2.1897, 2.1092, 3.2903, 3.5421, 2.4439, 3.4011, 1.9586 (table 1). However, three out of nine compounds exhibited powerful interaction with His51, Asp75 and Ser135 (fig. 1).

ADMET analysis

The selected nine compounds followed the Lipinski's rule of five and were subjected to ADME analysis to evaluate their drug like properties (table 2).

The 2D and 3D interaction of papaya compound with serine proteases (fig. 2) gave better understanding of the interaction with His51, Asp75 and Ser135 residue of dengue serine proteases. The compound with the code name F had the highest docking score. Similarly, compounds with code name H and I had respectively high docking score and great bonding affinity with His51, Asp75, Ser135 and residues. Therefore, three compounds namely epigallocatchin, catechin and protocatechuric acid were found to be more effective for inhibition of NS2B, NS3 and NS5 serine proteases of dengue virus.

FTIR Values of Methanol (ME) extract of C. papaya

The ME extract of papaya showed characteristic absorption bands at 3316.81cm^{-1}, 2947.95cm^{-1}, 2836.44 cm^{-1}, 2360.02cm^{-1}, 1648.25cm^{-1},1406.86cm^{-1},1112.88cm^{-1} and 562.38cm^{-1} (fig. 2).

Antioxidant activity

Papaya fruit showed maximum DPPH scavenging activity (86.5%) followed by leaves (82.2%) which shows its potential to be used as antioxidant agent (table 3).

Antimicrobial activity

The extract of *C. papaya* leaf and fruit has shown promising antimicrobial activity against all the pathogens used. The results are given in table 4.

DISCUSSION

Different approaches like structure based virtual screening, scaffold hopping, ligand based virtual screening, non-competitive binding, pepdidomimetics and small compounds libraries have been used to find out the inhibitors for dengue virus NS2B, NS3 and NS5 proteins. Several peptides, synthetic small molecules, cyclopeptides and natural inhibitors of DV NS2/NS3 have been reported but all these inhibitors may have high toxicity and weak bonding with the DV NS2/NS3 protease (Idrees *et al.,* 2014) but only seven of them interacted with DV NS2B/NS3. However, results need *in vitro* investigation and optimization to confirm their efficacy (Wei *et al.,* 2007; Powell *et al.,* 2013). The current study was designed for computational screening of *C. Papaya phytochemicals* against dengue NS2B, NS3 and NS5. About 900 bioactive compounds of *C. Papaya* were docked with the active site of Dengue NS2B/NS3 and nine compounds like 1-Hydroxy-2-propanone, 2-methyl-propanoic acid, Baicalein, 2-Methyl-butanoic acid, Epigallocatchin, Fisetin, Genistein, Catechin and Protocatechuric acid had potential interaction with His51, Asp75, and Ser135. The compounds were ranked on the basis of S-Score, RMSD value and bonding interaction with active residues (Araújo *et al*., 2012). Nine compounds A, B, C, D, E, F, G, H and I showed significant interaction with active residues of dengue NS2B/NS3.

The comparative determination of antioxidant activities of different parts of papaya was a novel approach to establish *in-vivo* studies for these compounds. Previous studies were focused on exploring the antioxidant potential of only single part of papaya (Ma *et al.,* 2017; Jarisarapurin *et al.,* 2019). The results showed the higher antioxidant activity in fruit and leaf extracts than others in contrast to Maisarah *et al.* (2013) which showed the highest DPPH scavenging activity than the extracts of fruits and seeds.

The present work reports antimicrobial potential of different parts of papaya plant. The findings of antimicrobial activity suggested the higher efficacy of leaves extract of papaya against all microbes used. Asghar *et al.* (2016) also conducted the same kind of work by using different solvents for extracts preparation and reported higher antibacterial activity in papaya pulp following the leaves extract.

CONCLUSION

Exploration of bioactive compounds from medicinal plants is an emerging field in pharmaceutical research. The present study was aimed to explore the anti-dengue potential of *C. papaya* through molecular docking, antimicrobial activity and antioxidant activity. The molecular docking of all the bioactive compounds of papaya was executed against dengue virus NS2B, NS3 and NS5 serine proteases. Nine compounds out of 900 were screened out and showed highest docking scores. Three compounds out of these nine, namely epigallocatchin, catechin and protocatechuric acid have the highest interaction with the serine proteases of dengue virus. These compounds have the strong binding affinity with His51, Asp75 and Ser135 residues of serine proteases. The antimicrobial activity resulted in a very sensitive zone of inhibition while DPPH scavenging activity also showed very promising results. Therefore, these compounds can be commercially applied for medical formulation against dengue virus.

ACKNOWLEDGEMENT

The authors acknowledge Higher Education Commission of Pakistan for funding this research under Grant No. 5696/Punjab/NRPU/R&D/HEC/2016.

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