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Computer Aided Screening of Phytochemicals from Garcinia against the Dengue NS2B/NS3 Protease

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Abstract:
Dengue virus NS2/NS3 protease because of its ability to cleave viral proteins is considered as an attractive target to screen antiviral agents. Medicinal plants contain a variety of phytochemicals that can be used as drug against different diseases and infections. Therefore, this study was designed to uncover possible phytochemical of different classes (Aromatic, Carbohydrates, Lignin, Saponins, Steroids, Tannins, Terpenoids, Xanthones) that could be used as inhibitors against the NS2B/NS3 protease of DENV. With the help of molecular docking, Garcinia phytochemicals found to be bound deeply inside the active site of DENV NS2B/NS3 protease among all tested phytochemicals and had interactions with catalytic triad (His51, Asp75, Ser135). Thus, it can be concluded from the study that these Gracinia phytochemicals could serve as important inhibitors to inhibit the viral replication inside the host cell. Further in-vitro investigations require confirming their efficacy.

Keywords: Dengue virus (DENV), NS2/NS3 Protease, Medicinal Plants, Phytochemicals, Inhibitors, Molecular docking, Catalytic traid

Background:
Dengue viral infection has become a serious issue about human health **[1].** Dengue virus (DENV) belongs to *Flaviviradae* family **[2].** In recent years, it has been reported in more than 100 countries **[3],** Asia, Central and South America and Africa are the major affected regions with this infection **[4, 5].** According to recent studies, it has been found that NS3 protein is most important non-structural protein involved in DENV infection. This region is named as NS3pro and its activity depends on a cofactor named as NS2B. These two collectively forms a complex known as NS2B-NS3pro complex. Any disruption in functional activities of NS2B-NS3pro complex inhibits viral replication **[6].** Different compounds like, chemistry-derived benz[d]isothiazol-3(2H)-one derivatives (2-[2-Phenyl-1-(2-p-cyanophenyl-1,3,4-oxadiazol-5-yl)ethyl]-1,2-benzisothiazol-3(2H)-one (a), 2-[2-Phenyl-1-(2-p-fluorophenyl-1,3,4-oxadiazol-5-yl)ethyl]-1,2-benzisothiazol-3(2H)-one (b), 2-[2-Phenyl-1-(2-p-methoxyphenyl-1,3,4-oxadiazol-5-yl)ethyl]-1,2-benzisothiazol-3(2H)-one(c),2-[2-Phenyl-1-(2-phenyl-1,3,4-oxadiazol-5-yl)ethyl]-1,2-benzisothiazol-3(2H)-one (c), 2-[2-Phenyl-1-(2-p-chlorophenyl-1,3,4-oxadiazol-5-yl)ethyl]-1,2-benzisothiazol-3(2H)-one) (d) **[7],** SK-12 **[8]** and kalata B1 analogues have been reported as DENV NS2B-NS3 protease inhibitors **[9].** But there is no effective drug available for the treatment of DENV infection yet **[10].** Therefore, present study has been designed to increase the spectrum of DENV NS2/NS3 protease inhibitors. Total 940 phytochemicals of different classes (Aromatic, carbohydrates, lignin, saponins, steroids, tannins, Terpenoids, Xanthones) possessing antiviral activity against Dengue virus were computationally screened against Dengue virus. The main idea behind this study was to target the sites of Dengue virus NS2B/NS3 to identify novel phytochemicals that could inhibit the DENV infection. The results of this study will offer useful information about drug development.

Methodology:
940 phytochemicals have been docked against DENV NS2B/NS3 protease using the Molecular Operating Environment (MOE) software package.

Retrieval and refinement of receptor

3D structure of DENV NS2B/NS3 protease was retrieved from the Protein Data Bank (PDB) using PDB ID: 2FOM. To refine the structure, water molecules were removed from the structure and 3D protonateion was done by using MOE to change the state into ionization level. Moreover, energy minimization was done using default parameters.

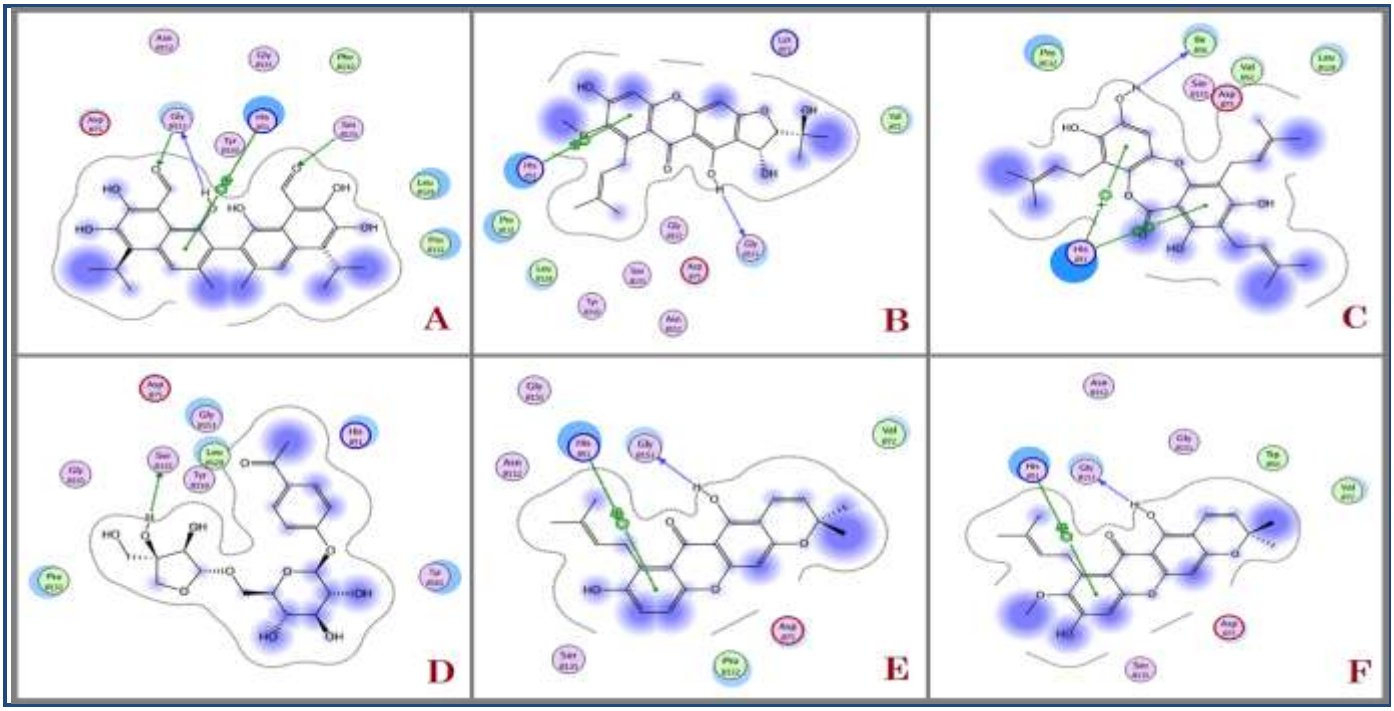

Figure 1: A) (-)-Gossypol interaction with DENV NS2B/NS3pro; **B)** Mangostenone C interaction with DENV NS2B/NS3pro; **C)** Garcidepsidone A interaction with DENV NS2B/NS3pro; **D)** 4-hydroxyacetophenone 4-O-(6'-O-beta-D-apiofuranosyl)-beta-D-glucopyranoside interaction with DENV NS2B/NS3pro; **E)** Demethylcalabaxanthone intraction with DENV NS2B/NS3pro; **F)** Mangostanin interaction with DENV NS2B/NS3pro.

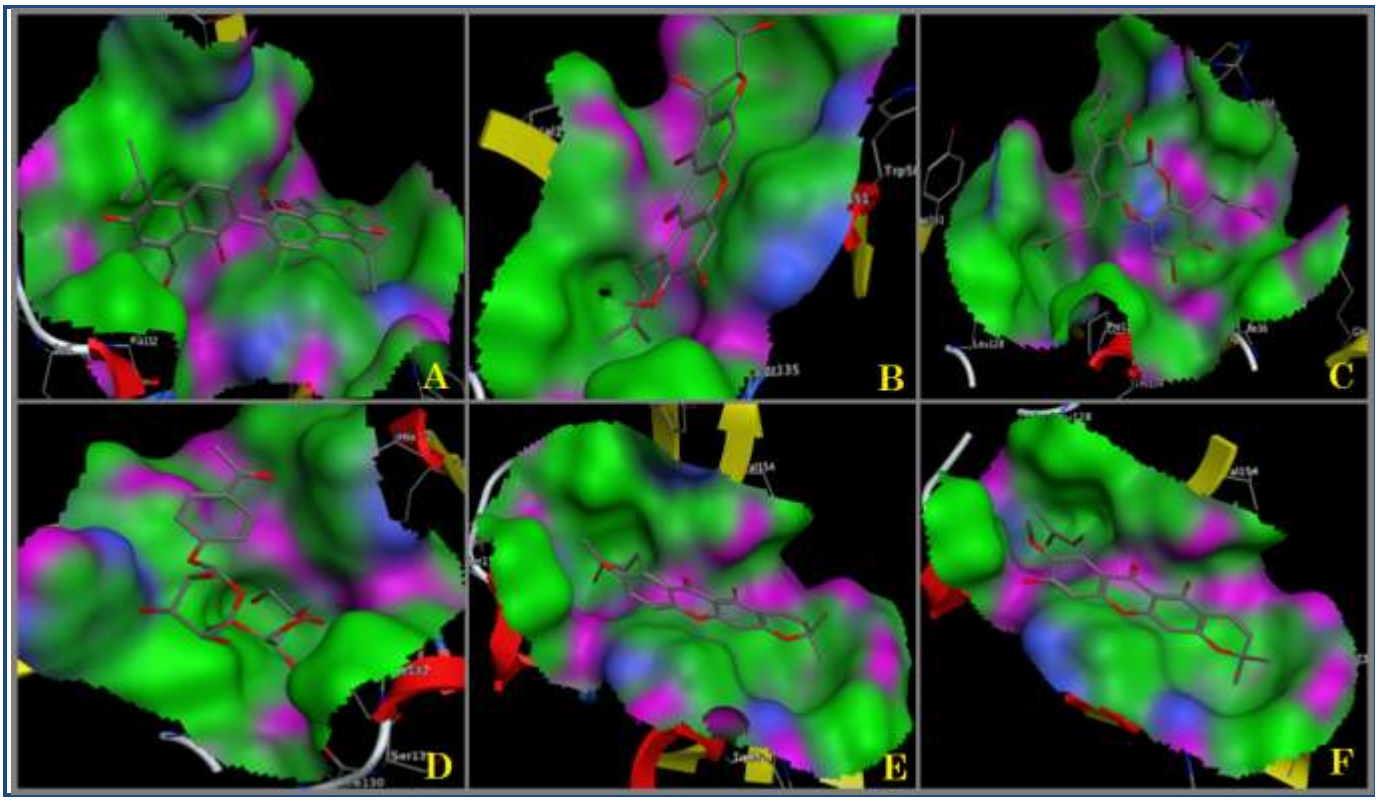

Figure 2: A) Binding mode of (-)-Gossypol with receptor pocket; **B)** Binding mode of Mangostenone C with receptor pocket; **C)** Binding mode of Garcidepsidone A with receptor pocket; **D)** Binding mode of 4-hydroxyacetophenone 4-O-(6'-O-beta-D-

apiofuranosyl)-beta-D-glucopyranoside with receptor pocket; **E)** Binding mode of Demethylcalabaxanthone with receptor pocket,; **F)** Binding mode of Mangostanin with receptor pocket.

Ligand database preparation

940 phytochemicals of 8 classes were retrieved from MAPS database **[11].** Energy minimization was done by using default parameters of MOE. Phytochemicals were stored in .mol format and made a database in .mdb format by using MOE.

Molecular Docking

Active site residues were find out by using site finder option of MOE. Docking algorithm of MOE was used to dock phytochemicals database with the catalytic triad (His 51, Asp 75, Ser 135) of DENV NS2/NS3 protease. The parameters were set as (Re-scoring function: London dG, placement: triangle matcher, Retain: 10, Refinement: Force field, and Re-scoring 2: London dG). Top conformation for each phytochemical was selected on the basis of minimum S score and were further evaluated to study the interactions by using ligx option of MOE.

Results & Discussion:

Molecular Docking

DENV has four serotypes **[12]** but the binding site of NS2B-NS3 has same substrate specificities thus, any inhibitor against the binding pocket of NS2/NS3 protease could work against all the serotypes **[13].** 940 phytochemicals were computationally screened. MOE provided ten conformations for each phytochemical. The negative and low score for any ligand shows favorable interactions between ligand and the receptor protein. Hence, top conformation for each phytochemical having minimum S score were further analyzed for interaction analysis.

Interaction analysis

Through intraction analysis six phytochemicals were found that bound deeply inside active site pocket and successfully blocked DENV NS2B-NS3 protease and may serve as important drug candidates against NS3 protease. Beside minimum S score, (-)-Gossypol also had potential interactions with His51, Ser135 and strong hydrophobic contact with Asp75 of catalytic triad, thus it placed at the top. All other phytochemicals (Mangostenone C; Garcidepsidone A; 4-hydroxyacetophenone4-O-(6'-O-beta-D-apiofuranosyl)-beta-D-glucopyranoside; Demethylcalabaxanthone; Mangostanin) also have potential interaction and significant hydrophobic contact with active residues of catalytic triad and thus, it can be concluded that these phytochemicals could be use as potential drug against Dengue virus NS2B/NS3 Protease. Details about selected phytochemicals and interacting residues of the DENV NS2B/NS3 Protease with phytochemicals are shown in **Table 1 (see supplementary material)**. Interactions between Dengue virus NS2B/NS3 Protease catalytic triad and selected phytochemicals are shown in **Figure 1**. Binding mode of ligands with receptor pocket that shows how deeply ligand bind inside receptor pocket is shown in **Figure 2**.

Conclusion:

Dengue is a global health challenge, no vaccine has been developed to target this disease yet. Improved strategies are required to develop drug candidates that can inhibit DENV infection. Present study focused on the screening and docking of medicinal plant phytochemicals against NS2/NS3 protease. This study has found possible binding of phytochemicals which interacts with the active sites of DENV NS2/NS3 protease. Moreover, It was found that phytochemicals of genus *Garcinia* have greater ability to inhibit DENV replication. This study will be helpful in drug designing before synthesizing and testing them. Thus, it can be concluded from this study that *Garcinia* phytochemicals could serve as future drug candidates.

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References:

[1] Idrees S & Ashfaq UA, *Genet Vaccines Ther*. 2012 **10:** 6 [PMID: 22929369]
[2] Weaver SC & Vasilakis N, *Infect Genet Evol*. 2009 **9:** 523 [PMID: 19460319]
[3] http://wwwwhoint/mediacentre/factsheets/fs117/en/
[4] Das S *et al. J Clin Microbiol*. 2008 **46:** 3276 [PMID: 18685000]
[5] Fatima Z *et al. BMC Microbiol*. 2011 **11:** 200 [PMID: 21906394]
[6] Tiew KC *et al. Bioorg Med Chem*. 2012 **20**: 1213 [PMID: 22249124]
[7] Gao Y *et al. Bioorg Med Chem*. 2010 **18:** 1331 [PMID: 20042339]
[8] Pambudi S *et al. Biochem Biophys Res Commun*. 2013 **440**: 393 [PMID: 24070610]
[9] Idrees S & Ashfaq UA, *Asian Pac J Trop Biomed*. 2013 **3**: 232 [PMID: 23620845]
[10] Lengauer T & Rarey M, *Curr Opin Struct Biol*. 1996 **6:** 402 [PMID: 8804827]
[11] Ashfaq UA *et al. Bioinformation* 2013 **9:** 993 [PMID: 24391364]
[12] Khan AM *et al. PLoS Negl Trop Dis*. 2008 **2:** 272 [PMID: 18698358]
[13] Li J *et al. J Biol Chem*. 2005 **280:** 28766 [PMID: 15932883]

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Supplementary material:

Table 1: Phytochemicals detail and their interaction with DENV NS2B/NS3 protease

Plant Name	Phytochemical Class	Phytochemical name	S Score	RMSD value	Interacting residues (hydrogen bonding)	Closer contact residues
Gossypium hirsutum	Terpenoid	(-)-Gossypol	-9.4293	1.9010	His51, Ser135, Gly153,	Asp75, Leu128, Pro132, Tyr150
Garcinia mangostana	prenylated xanthones	Mangostenone C	-10.5737	1.1856	His51, Gly153	Asp75, Ser135, Leu128, Pro132, Val72, Ly73, Tyr150
Garcinia parvifolia	Aromatic	Garcidepsidone A	-10.2918	1.1454	His51, lle36	Asp75, Ser135, Leu128, Val52, Pro132
Salvia officinalis	flavone glycosides	4-hydroxyacetophenone 4-O-(6'-O-beta-D-apiofuranosyl)-beta-D-glucopyranoside	-9.2620	1.9114	Ser135	Asp75, His51, Leu128, Gly153, Tyr161, Pro132
Garcinia vieillardii	xanthones	Demethylcalabaxanthone	-10.2745	1.6736	His51, Gly153	Asp75, Ser135, Pro132, Val72
Garcinia mangostana	xanthones	Mangostanin	-10.6745	1.0520	His51, Gly153	Asp75, Ser135, Trp50, Val72, Gly151