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RESEARCH ARTICLE

Screening of *Tephrosia purpurea* Compounds as Potential Inhibitor for Dengue Virus NS2B / NS3 Protease

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ABSTRACT

Dengue is a mosquito-borne viral disease caused by dengue virus and the infection becomes a serious health concern globally because of the high mortality rate. Due to the high prevalence of dengue viral infections and having no specific treatment, the development of novel antiviral agents is essential to control of dengue virus. Antiviral substances obtained from natural products and are commonly prescribed for the dengue patients but there are no scientific evidences for its activity against dengue virus. Therefore, the present study was undertaken to investigate the anti-viral activity of compounds present in the root of *Tephrosia purpurea* against non-structural proteins of dengue virus (DENV) using spectroscopy and computational molecular docking strategies. The selected plant *T. Purpurea* was partially purified and tested against dengue vectors. The active plant extracts were further purified and characterized by FTIR, GC-MAS and NMR spectra. Resulting four larvicidal compounds (Tephrosin, Purpurin, Deguelin and Rotenone) were identified and used to molecular docking for prediction of predominant binding mode of a ligand with 3D structure of NS2B/NS3 protease that is considered a key technique. The energy minimized 3D structures of selected four compounds were docked with NS2B/NS3 protease using HEX 6.8 docking software. Therefore, the enzyme NS2B/NS3 used as receptor and the chemical compounds were act as ligand molecule. The present results revealed that four compounds showed high inhibitory activity against DENV NS2B/NS3 protease. These findings conclude that these selected compounds could serve as antiviral drugs for dengue infections.

KEYWORDS

Tephrosin, Purpurin, Deguelin, Rotenone, DENV NS2B/NS3 Protease and Molecular docking

INTRODUCTION

Dengue is a mosquito-borne disease caused by dengue virus (DENV) and there are four distinct serotypes, DENV I – IV. DENV belongs to the family *Flaviviridae,* which causes a spectrum of illness ranging from an apparent infection to moderate febrile illness, severe and fatal hemorrhagic disease. The most common infection produces the classical dengue fever (DF), the most common infection produces the classical dengue fever (DF), which is characterized by a sudden onset of rash, high fever, headache and backache. The main clinical manifestations namely, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), are responsible for high morbidity and mortality rates every year in especially southern districts of Tamil Nadu[1,2,3]. DENV is a positive-stranded encapsulated RNA virus and is composed of a single polyprotein which is then proteolytically cleaved into three structural protein genes are nucleocapsid or core (C) protein, a membrane-

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associated (prM) protein, an enveloped (E) glycoprotein and seven non-structural proteins are NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5[4,5].

The structural proteins play a role in the formation of viral particle[6,7] and non-structural proteins form the replication complex which includes the NS3 protease with its NS2B as cofactor which is required to cleave the polyprotein. This NS2B/NS3 protease complex is required for viral replication. Therefore, it serves as a promising target for anti-dengue viral drug development. The 3D structure of the NS3 protease domain has been determined, but the structural determinants necessary for activation of the enzyme by the NS2B cofactor have been characterized only to a limited extent. The NS2B/NS3 two-component protease mediates cleavage in the nonstructural region of the viral polyprotein at the NS2A/NS2B, NS2B/NS3, NS3/NS4A, and NS4B/NS5 junctions. However, the population of dengue vectors (the main vector *Aedes aegypti*, *Ae. albopictus* and *Ae. polynesiensis*) that are responsible for the increase in deaths due to dengue is on the rise. In view of the fact that natural products have been widely used for a long time in India to control pests, vectors, pathogens and to treat various disorders including infectious diseases. Recently, many researchers find out that the phytochemicals derived from plant sources might be alternative agents for the control of dengue vectors, because they have larvicidal, pupicidal, adulticidal and repellent activity[8,9,10,11,12,13]. Therefore, the present study was undertaken to evaluate the efficacy of *Tephrosia purpurea* L. root compounds to demonstrate their ability to dengue NS2B/NS3 protease binding using computational molecular docking strategies.

MATERIAL AND METHODS

Isolation of Plant Compounds

Tephrosia purpurea L. (Family: Fabaceae) root was collected from Sivakasi Taluk, India and identified with voucher specimen. The roots powdered materials were weighed and extracted over night in analytical grade methanol (MeOH) in the ratio 1:10 W/V over a magnetic stirrer. The MeOH extract was filtered and concentrated in a vacuum evaporator at 45°C under low pressure and ultra-filtration method. The extract was subjected to column chromatograph on silica gel (150 gm) eluted with two solvent system of cyclohexane to ethyl acetate (ratio 4:1 and 9:1) which resulted many fractions. The major mosquitocidal active fractions were further purified using varying systems of dichloromethane, cyclohexane and methanol to obtain eight fractions based on solvent polarities.

Identification of Phytocompounds

Isolated four plant compounds were identified by FTIR and NMR spectra. GC-MAS analysis which was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20I auto sampler and gas chromatograph interfaced to a mass spectrophotometer. Interpretation of the mass spectrum of GC-MAS was conducted using the database of National Institute Standard and Technology (NIST) which consists of more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component inherent in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. Identified compounds; Tephrosin, Purpurin, Deguelin and Rotenone were used to dengue virus NS2B/NS3 protease blocking studies.

Molecular Modeling

The molecular model generation needs query sequence; it was retrieved from NCBI database which specific identification (DENV – NS2B/NS3 protease) with Drug bank. The protein sequence of NS3 was used to found suitable template coordinates which have already crystallographic structure in PDB from NCBI search generated a template, mutant type of same protein in same virus and their PDB id is 2FOM. (Chain A, Insertion Mutant E173gp174 of The NS3 Protease-Helicase from Dengue Virus). Preparation of template-sequence alignment and generation of model structure NS3 protein were done in Hex 6.8 using template and query protein sequence.

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The 3D structure of NS2B and NS3 protease enzyme was retrieved from the PDB database and the structures of four compounds from the GC-MS results and was retrieved from Drug bank and PUBCHEM through NCBI website. The energy minimized 3D structures of plant compounds were docked with NS2B and NS3 protease enzyme using HEX docking software. The enzyme NS2B and NS3 used as receptor and the chemical compounds were act as ligand molecule. The effectiveness of four compounds can be determined via the docking studies by calculating their energy minimization value. Before, the compounds PDB file were downloaded from drug bank and uploaded in the Hex as ligand files. After that the docking control, parameters and models to display were set to the receptor and ligand molecule. The output was set to predict 200 solutions. Because, the final docked structure, was completely energy minimized with lowest energy conformation. The lowest energy minimized value is the most suitable for drug stability.

RESULTS AND DISCUSSION

The preliminary results pointed out that ethyl acetate (EtOAc) extract of the *T. purpurea* root was more active for *Aedes aegypti* than the other extracts. Therefore, the extract was further purified and compounds identified by column chromatography and GC-MS analysis. The plant active compounds; tephrosin, purpurin, deguelin and rotenone were also analyzed for the drug like properties. Before, unknown compounds were identified by compared with the spectrum of the known component inherent in the NIST library. Therefore, the four known compounds only were used to dengue virus NS2B/NS3 protease blocking studies. The structural details and the smiles notation of the selected four compounds were retrieved from PubChem / Drug bank database (http://pubchem.ncbi.nlm.nih.gov/). The Smiles notation of four compounds was obtained from Drug bank/PubChem were subjected to energy minimized 3D structure file. After building the structures of plant chemical compounds successfully, geometry optimization and energy minimization were completed. The virus non-structural proteins, NS3-mediated processing of the protein junctions is essential for viral replication and therefore provides an attractive target for development of antiviral agents[14,15]. Then molecular docking was performed for the dengue NS2B/ NS3 protease enzyme obtained from Protein Data Bank (2FOM) with the four chemical compounds taken as ligands using HEX 6.3 tool (Table 1). The energy minimization process was performed for 100 - 2000 solutions using the Hex software. From the docking result various conformations of the ligands were analyzed (Figure 1 - 3).

The docking of the ligands was carefully observed for its conformation and docking energy. The ligands having Drug bank database ID 107935 (Deguelin) shows binding affinity and their E-values predicted is -305.9. The binding position of the highest docking energy was observed for the compound, deguelin with the NS2B/NS3 protease (2FOM). The conformations and Energy minimization values for the docked compounds were analyzed (Table 2). Deguelin has the highest docking score along with more number of hydrogen bonds formed, whereas tephrosin and purpurin had good docking score with hydrogen bond interaction. These findings suggest that better than leaves of *Broussonetia papyrifera, Trigonella foenum-graecum* and *Carica papaya* compounds score[16,17]. The other three ligands, it showed the different conformation after docking. The rest of three ligands are tephrosin, purpurin, and rotenone were observed to share a similar position in the protein also showed similar conformation and their E values are -258.1, -195.4 and -240.9 respectively. This docking result suggests that different ligands bind to the same binding site under receptor conformations. The results of the present study were in correlated with the early reports of protein-ligand binding interaction study by performing docking of the ligands that were found to be competitively inhibiting the activities of the DENV NS2B/NS3 protease[18,19]. Docking to different ligands conformations also due to change of a receptor-specific conformations. Therefore, structural based drug designing protein-ligand interaction plays a remarkable role. It has been clearly manifested

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that the approach employed in this study is victorious in finding best possible dengue inhibitors from root compounds of *T. purpurea.* Therefore, the prediction of these selected compounds from the Docking study would help to screen the compound against dengue virus and other flavivirus family and also this drug may used to inhibit dengue virus replication.

Further, *in vitro* and *in vivo* studies on dengue virus are necessary to confirm their efficacy and to evaluate their drug potency.

Table 1: Chemical Properties of *T. purpurea* root compounds retrieved from PubChem Database

Chemical properties	***T. Purpurea* compounds**			
	Tephrosin	**Purpurin**	**Deguelin**	**Rotenone**
PubChem CID	114909	6683	107935	6758
Molecular Formula	$C_{23}H_{22}O_7$	$C_{14}H_8O_5$	$C_{23}H_{22}O_6$	$C_{23}H_{22}O_6$
Molecular Weight	410.42 g/mol	256.21g/mol	394.14g/mol	394.42 g/mol
Log P value	3	2.9	3.7	4.1
H bond acceptors	1	5	6	6
H donors	7	3	0	0

Table 2: The selected four compounds energy values from PDB database

E. values	***T. Purpurea* compounds**			
	Tephrosin	**Purpurin**	**Deguelin**	**Rotenone**
Energy minimization	-258.1	-195.4	**-305.9**	-240.9
Energy maximization	-168.0	-146.7	**-245.8**	-170.9

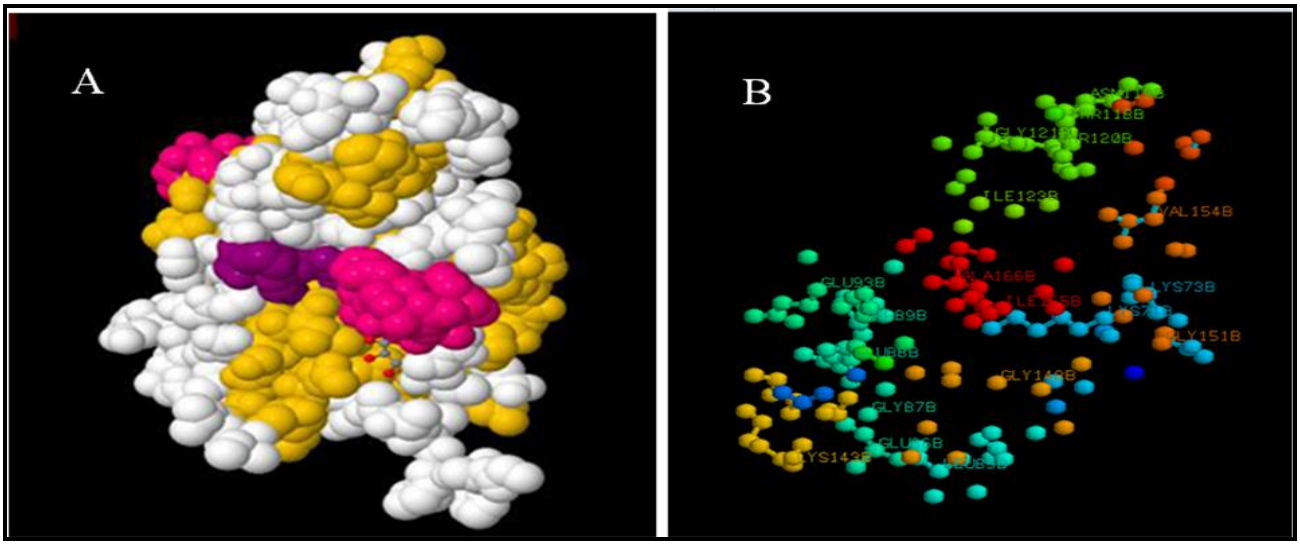

Figure 1: Dengue virusNS2B/NS3 protease retrieved from PDB database using Metapocket 2.0 software. A) 3D structure of NS2B/NS3 protease, ligand binding sites for 2 FOM on protein surface. B) Potential binding atoms of all the binding sites

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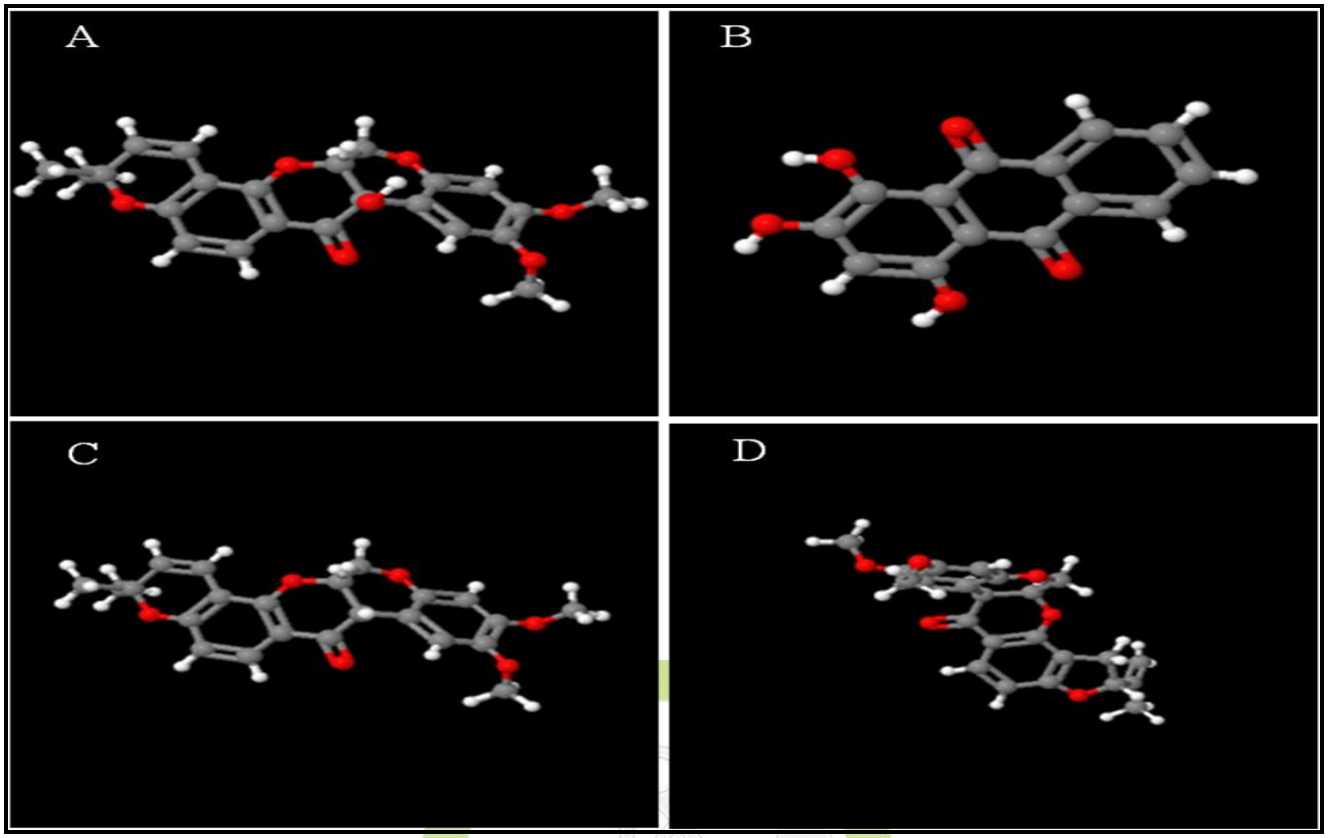

Figure 2: *T. purpurea* root compounds retrieved from Pub Chem Database and used as Ligands. A) Tephrosin, B) Purpurin, C) Deguelin and D) Rotenone

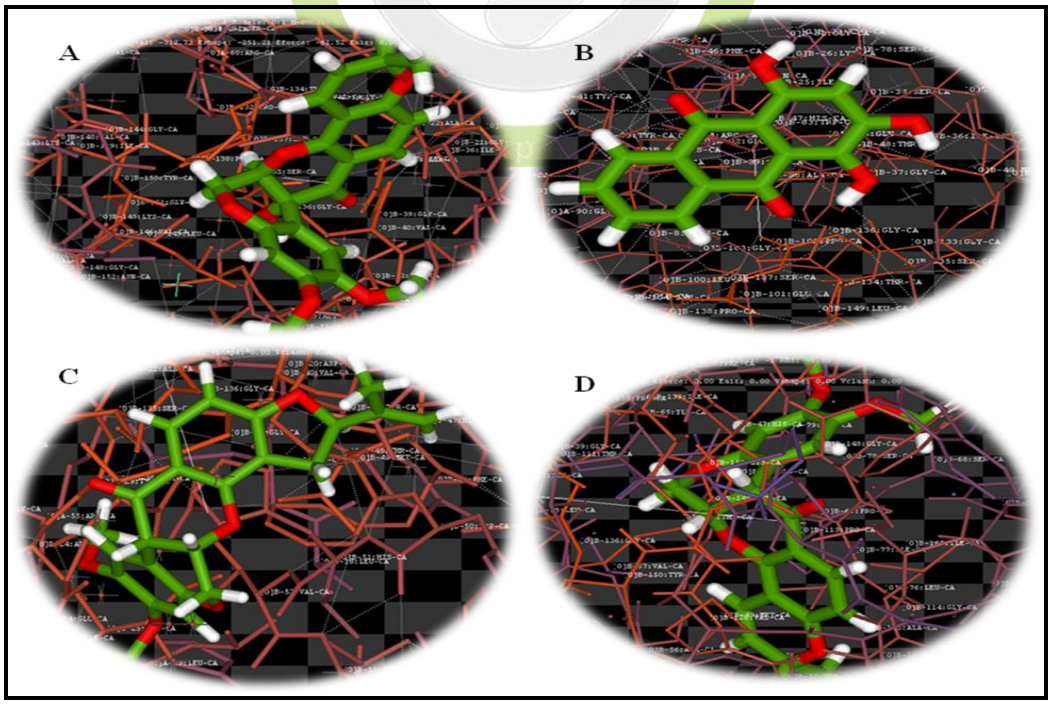

Figure 3: Hydrogen bond interaction of the NS2B/NS3 protease (2FOM) active sites with *T. purpurea* root compounds using HEX docking software; A) NS2B/NS3 protease with Tephrosin, B) NS2B/NS3 protease with Purpurin, C) NS2B/NS3 protease with Rotenone, D) NS2B/NS3 protease with Deguelin

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