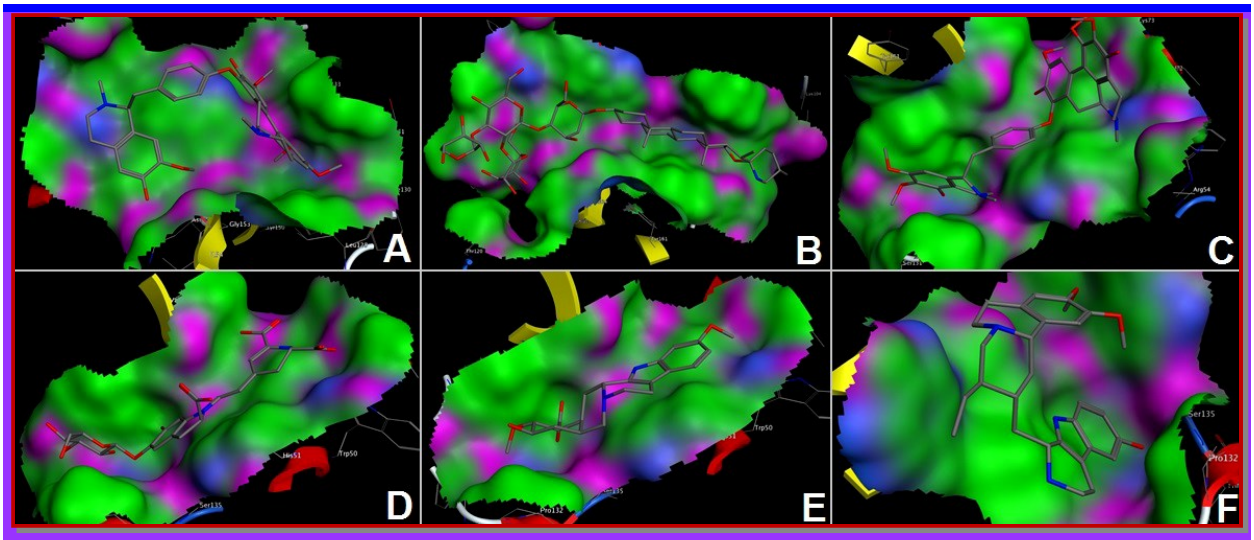

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Potential of plant alkaloids as dengue NS3 protease inhibitors: Molecular docking and simulation approach

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Abstract

Dengue infection has become a worldwide health problem and infection rate is increasing each year. Alkaloids are important phytochemicals of medicinal plant and can be used as vaccine candidates for viruses. Therefore, present study was designed to find potential alkaloids inhibitors against the Dengue virus NS2B/NS3 protease which can inhibit the viral replication inside the host cell. Through molecular docking it was investigated that most of the alkaloids bound deeply in the binding pocket of Dengue virus NS2B/NS3 protease and had potential interactions with catalytic triad. Five alkaloids (6′-desmethylthalifaboramin; 3,5-dihydroxythalifaboramine; Betanin; Reserpic acid and Tubulosine) successfully blocked the catalytic triad of NS2B/NS3 protease and these alkaloids can serve as a potential drug candidate to stop viral replication. It can be concluded from this study that these alkaloids could serve as important inhibitors to inhibit the replication of DENV and need further *in vitro* investigations to confirm their efficacy and drug ability.

Introduction

Dengue infection has become a global health problem and this appalling disease has affected almost 2.5 billion people (Idrees and Ashfaq, 2012) with an estimated 25,000 deaths per year (Hakim et al., 2011). Recent studies have shown that more than 100 countries and about 50-100 million people are being affected with this appalling disease. Asia, Central and South America and Africa are the major affected areas relatively (Das et al., 2008). Dengue virus (DENV) is a member of *Flaviviradae* family containing four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) (Weaver and Vasilakis, 2009). Among humans *Aedes aegypti* and *Aedes albopictus* are two mosquitoes act as vector for the transmission of DENV infection (Thomas et al., 2003). The DENV genome is of 11 kb and encodes a polyprotein. This polyprotein is cleaved into 10 viral proteins including three structural and seven non-structural proteins. The order of these proteins is capsid, premembrane, envelope protein, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Non-structural proteins are mainly involved in viral replication (Chambers et al., 1990).

According to recent studies, it has been found that NS3 has a serine protease domain at the N terminal region and its activity depends on its interaction with cofactor (NS2B). These two forms a complex called NS2B-NS3pro complex. This complex is very important because it has ability to cleave viral proteins. Any disruption in functional activities of this region results into the inhibition of viral replication. Hence, to screen and evaluate effects of different drug candidates, NS2B-NS3 complex is considered an important target (Rothan et al., 2012). Currently, there is no vaccine and effective drug available for the treatment of DENV infection (Idrees and Ashfaq, 2012).

Medicinal plants contains naturally occurring phyto-

chemicals (Calixto, 2000). These phytochemicals defend not only plants but also can protect humans and animals against different diseases (Kubmarawa et al., 2008). Medicinal plants contain variety of phytochemicals such as organosulfur compounds, limonoids, furyl compounds, alkaloids, polyines, coumarins, thiophenes, peptides, flavonoids, terpenoids, polyphenolics and saponins, revealing therapeutic functions due to scavenging, hampering viral entry and DNA\ RNA replication against diverse range of viruses (Idrees et al., 2013). Alkaloids are important phytoche-micals having anti-viral activity. Different studies have shown that alkaloids can play a pivotal role in viral diseases treatment (Watson et al., 2001). Medicinal plants are preferred over conventional treatment because they have low cost, multiple target activities, negligible side-effects and little probable to cause resistance (Jassim and Naji, 2003).

Recent computational techniques have opened new doors to drug development studies. Prediction of predominant binding mode of a ligand with a protein of known three-dimensional structure (Molecular docking) is considered as important technique in drug designing and screening of novel antiviral compounds against challenging diseases (Lengauer and Rarey, 1996). Therefore, this study has been designed to screen 1300 alkaloids of more than 80 antiviral medicinal plants against Dengue virus NS2B/NS3 protease using *in silico* techniques. The main theme of this study was to target the hydrophobic pockets of Dengue virus NS2B/NS3 Protease to screen novel alkaloids that could help in inhibition of the DENV infection. The result of this study will offer useful information about drug development and would help in computer aided screening of the drugs against DENV infection.

Materials and Methods

In this study alkaloids have been docked against Dengue virus NS2B/NS3 protease. Docking was carried out using the Molecular Operating Environment (MOE) software package (ul qamar et al., 2014).

Ligand database preparation

A literature survey was performed to find alkaloids from antiviral medicinal plants, which were found effective against viral diseases specially against Dengue Virus. Chemical structures of alkaloids were downloaded from MAPS Database (Ashfaq et al., 2013), PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Pubchem (http://www.ncbi.nlm.nih.gov/pccompound) and Zinc Data-base (Irwin et al., 2005). Structures were stored in .mol format. All the downloaded structures were optimized by adding hydrogen atoms using MOE software. Energies of selected molecules were minimized with parameters (gradient: 0.05, Force Field: MMFF94X, Chiral Constraint and Current Geometry). All these alkaloids were then saved in .mdb database which was further used for docking.

Refinement of receptor protein

Three-dimensional (3D) structure of the Dengue virus NS2B/NS3 protease was retrieved from the Protein Data Bank (PDB) using PDB ID:2FOM (http://www.rcsb.org/pdb) and was optimized by removing water molecules, 3D protonaion and Energy minimization using Molecular Operating Environment (MOE, 2012). Moreover, energy minimization was done using parameters, Force Field: MMFF94X + Solvation, gradient: 0.05, and Chiral Constraint: Current Geometry. This minimized structure was used as receptor for docking studies.

Molecular docking

The docking algorithm of the Molecular Operating Environment software was used to dock ligand database with catalytic triad (His 51, Asp 75, Ser 135) of Dengue virus NS2/NS3 protease. The parameters were set; Re-scoring function: London dG, placement: triangle matcher, Retain: 10, Refinement: Force field, and Re-scoring 2: London dG. Docking program of MOE provides correct conformation of the ligand so as to obtain minimum energy structure. After docking, top and best conformation for alkaloids was selected on the basis of S score to further study the hydrogen bonding/ π-π interactions.

Drug scan

Drug scan of final selected alkaloids was performed by using the ligand properties checking tool of MOE to make sure that the compounds possess appropriate molecular properties to be a drug candidate.

Results

The 3D-structure of DENV NS2/NS3 protease was retrieved from PDB. The PDB ID of 3D-structure was 2FOM, which had resolution of 1.50 Å. All alkaloids were docked with the catalytic triad of Dengue virus NS2B/NS3 Protease.

Molecular Operating Environment software provided ten conformations for each alkaloid. All these conformations were sorted according to S score. Top six conformations for each alkaloid with minimum S score were selected for further analysis. 6′-Desmethylthalifaboramin was ranked at top conformation followed by 3-hydroxy-6′-desmethyl-9-0-methylthalifaboramine, 3,5-dihydroxythalifaboramine, betanin, reserpic acid and tubulosine respectively. Plant names from which alkaloids were derived, S score, RMSD value and detail about interacting residues shown in (Table I). Chemical structures of selected alkaloids have shown (Figure 1).

Table I

Plant names from which alkaloids were derived, S score, RMSD value and detail about interacting residues is shown

Plant name	Alkaloids	S Score	RMSD value	Interacting residues	Close contact residues
Thalictrum faberi	6′-Desmethyl-thalifaboramin	-12.24	2.21	His51	Asp75, Ser135, Leu128, Pro132, Gly135, Trp50, Arg54
Thalictrum faberi	3-Hydroxy-6′-desmethyl-9-0-methylthali-faboramine	-11.96	2.09	Asn152, Lys73	Asp75, Asp129, His51, Thr120, Leu128, Gly153, Ser131
Thalictrum faberi	3,5-Dihydroxythali-faboramine	-11.66	1.53	His51, Asn152	Asp75, Ser134, Arg54, Gly153, Pro132, Leu128, Val154, Ser135
Hylocereus polyrhizus, Amaranthus powellii, Boerhavia erecta	Betanin	-11.45	3.00	His51, Pro132, Gly151, Tyr150, Phe130,	Asp75, Leu128, Ser135, Trp50, Val72, Gly153
Rauwolfia vomitoria	Reserpic acid	-11.15	2.31	His51	Asp75, Ser135, Gly135, Pro132, Trp50, Val72, Phe130
Pogonopus speciosus, Alangium lamarckii	Tubulosine	-10.64	2.12	Ser135	Tyr161, Asp75, Gly135, His51, Leu128, Pro132, Tyr150

Table II

Molecular properties of flavonoids assessed through Ligand properties checking tool of MOE

Alkaloids	Molecular weight	Log P	TPSA	Hydrogen bond donor	Hydrogen bond acceptor	Lipinski's rule of five
6′-desmethylthalifaboramin	640.5	3.8	95.5	4	6	Suitable
3-hydroxy-6′-desmethyl-9-0-methylthalifaboramine	1035.3	-3.7	342.4	13	21	Not-suitable
3,5-dihydroxythalifaboramine	686.8	3.5	124.9	5	8	Suitable
Betanin	550.5	-3.7	249.4	8	13	Suitable
Reserpic acid	401.5	0.9	96.2	4	5	Suitable
Tubulosine	477.6	2.9	75.5	4	3	Suitable

Along with minimum S score, 6′-desmethylthalifaboramin also had potential interactions with His-51 and strong hydrophobic contact with Asp-75 and Ser-135 of catalytic triad and thus, it can be concluded that this alkaloid could use as potential drug against Dengue virus NS2B/NS3 Protease. All other alkaloids (3-hydroxy-6′-desmethyl-9-0-methylthalifaboramine; 3,5-dihydroxythalifaboramine; betanin; reserpic acid; tubulosine) also have potential interaction and significant hydrophobic contact with active residues of catalytic triad. Interacting residues of the DENV NS2B/NS3 Protease are shown in (Table I). Interactions between Dengue virus NS2B/NS3 Protease catalytic triad and alkaloids are shown in (Figure 2). Binding mode of ligands with receptor is shown in (Figure 3).

Drug scan

Final selected alkaloids were further analyzed to check Lipinski's Rule of Five using the Ligand properties checking tool of MOE which assessed the molecular properties and practicability of these compounds (Lipinski et al., 1997). The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion. These compounds were examined for their drug-suitableness and the results are shown in (Table II). Our results showed that all the alkaloids compounds used in this study fulfill the criteria of being drug candidates except 3-hydroxy-6′-desmethyl-9-0-methylthalifaboramine.

Discussion

Dengue is an appalling disease and requires urgent attention to develop new inhibitory compounds that could work against it. Genome of dengue encodes a single polyprotein which is cleaved into 10 viral proteins (Idrees et al., 2013). The cleavage of polyprotein precursor requires signal peptidase and NS3 serine protease which requires a cofactor named NS2B (Murthy et al., 1999). Dengue virus has four serotypes (Khan et al., 2008) but any inhibitor against the binding pocket of NS2/NS3 protease could work against all the serotypes (Li et al., 2005). Like other flaviviruses Dengue virus NS3 protease has been declared as

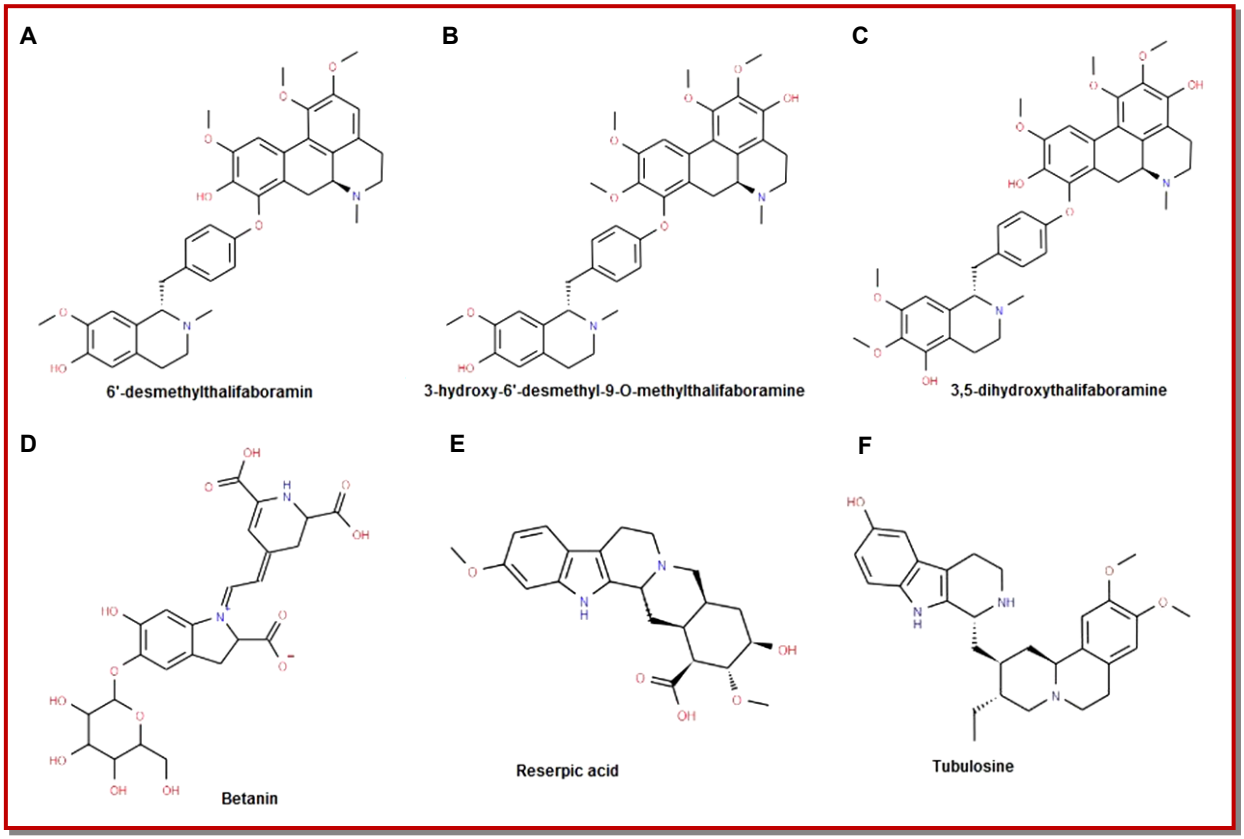

Figure 1: Chemical structures of selected alkaloids

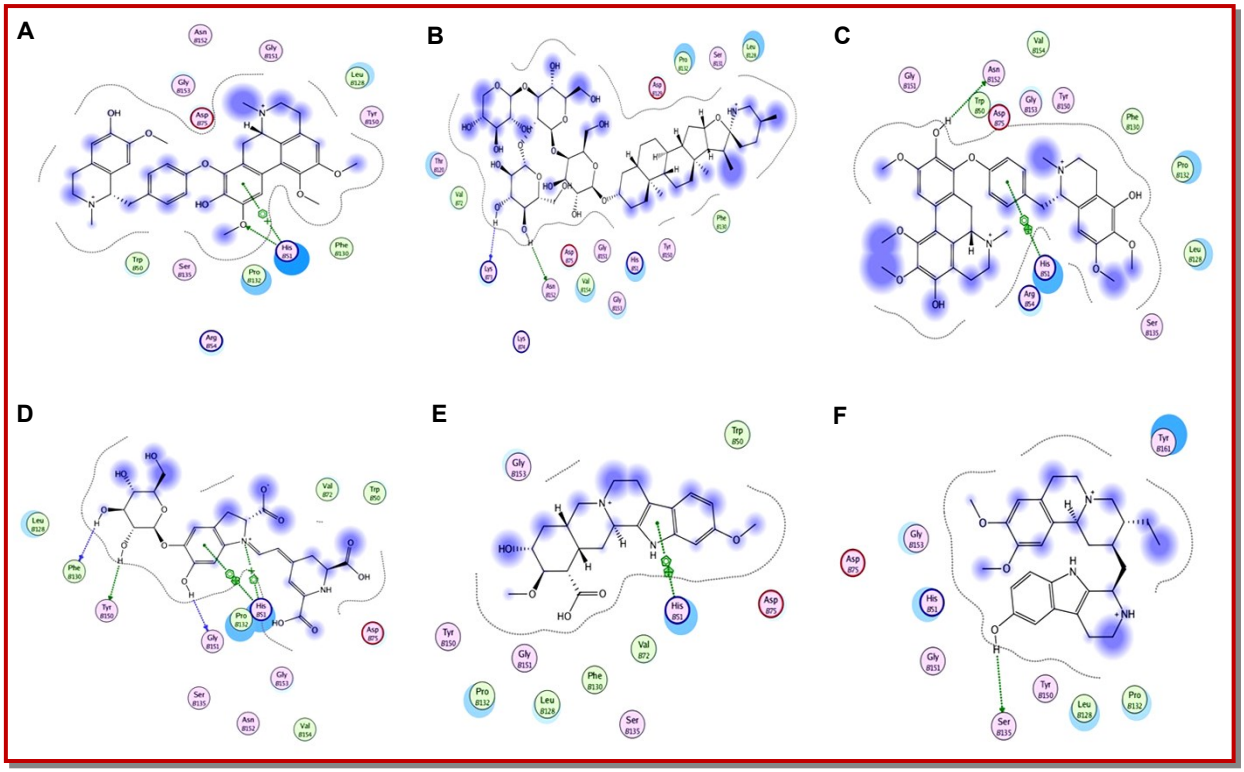

Figure 2: Binding interactions of alkaloids with active residues of Dengue virus NS2B/NS3 protease

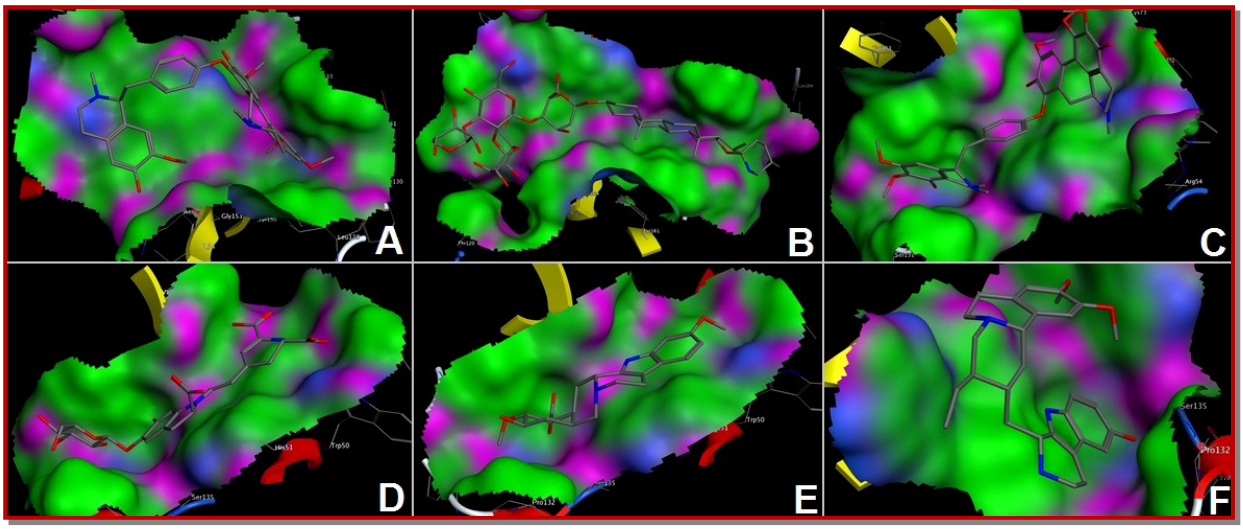

Figure 3: Docked alkaloids complexes with the binding pocket of dengue virus NS2B/NS3 protease

significant drug target. Catalytic triad is important in viral replication therefore, any disruption in it may block the replication of virus (Van Hell et al., 2009.

In recent research, computational techniques have enabled researchers to estimate the binding affinity of different molecules before their synthesis and evaluation in lab. Molecular docking used to find out the binding orientation of the small molecules against their targets. Thus, molecular docking is considered as important technique in drug designing and screening of novel compounds against this dreadful and challenging diseases (Lengauer and Rarey, 1996). The current study focused on the docking of the plants phytochemicals against NS2B-NS3 protease.

We examined the potential of 1300 alkaloids against Dengue virus NS2B-NS3 protease. Alkaloids were downloaded from different databases. In this study, 1300 alkaloids were docked with the catalytic triad of Dengue virus NS2/NS3 protease to find their affinity as inhibitors. Only top conformations after docking were selected on the basis of minimum S score. Our results showed potential and significant interactions of alkaloids with the active site residues of catalytic triad. Our results also showed that the final selected alkaloids fulfill the criteria of being drug candidates.

Through our study it was found that five alkaloids (6'-desmethylthalifaboramin; 3,5-dihydroxythalifaboramin; Betanin; Reserpic acid and Tubulosine) have potential interaction and significant hydrophobic contact with active residues of catalytic triad thus, it can be concluded that these alkaloid could use as potential drug against dengue virus NS2B/NS3 protease. Further study needs to be conducted on the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) of alkaloids proposed as a drug.

This study has discovered potential binding of alkaloids from medicinal plants *Thalictrum faberi, Hylocereus polyrhizus, Amaranthus powellii, Boerhavia erecta, Rauwolfia vomitoria, Pogonopus speciosus, Alangium lamarckii* with active residues of NS2/NS3 protease.

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Conflict of Interest

Authors declare no conflict of interest

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