Research Article

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Computational exploration of antiviral activity of phytochemicals against NS2B/NS3 proteases from dengue virus

Dang Virüs'den NS2B/NS3 proteazlarına karşı fitokimyasalların antiviral aktivitesinin hesaplamalı olarak araştırılması

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Abstract

Background: Dengue fever has emerged as a serious threat in Pakistan in the last few years with high morbidity rates and substantial mortality. In the present study, NS2B/NS3 protease from four dengue virus (DENV) serotypes have been targeted using 2350 phytochemicals from various medicinal plants.

Material and methods: The phytochemicals were subjected to docking against NS2B/NS3 proteases using AutoDock Vina focusing the binding site, and the binding energies were determined to screen the effectively docked phytochemicals. Pharmacological properties were also analyzed for all the phytochemicals using PreADMET web server.

Results: Binding affinities ranged from −4.0 to −9.8 kcal/mol and a threshold of −9.0 kcal/mol was applied for screening compounds. A total of 18 phytochemicals are screened for passing all evaluation criteria of a drug in which three were for DENV1-NS2B/NS3, five for DENV2-NS2B/NS3, six for DENV3-NS2B/NS3 and four for DENV4-NS2B/NS3. Erycristagallin and Osajin from *Erythrina variegate*, PapraineA from *Fumaria indica* and Aloe-Emodin from *Aloe vera* are the most potent inhibitors of NS2B/NS3 protease from DENV1, DENV2, DENV3 and DENV4, having binding affinities of −9.6 kcal/mol, −9.6 kcal/mol, −9.6 kcal/mol and −9.2 kcal/mol, respectively.

Conclusion: The effective drug-like properties of all 18 phytochemicals demonstrate the inhibition potential against dengue virus replication in human beings.

Keywords: Dengue virus; NS2B/NS3; Phytochemicals; Molecular docking; ADMET; Virtual screening.

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Özet

Amaç: Dang humması, son birkaç yılda yüksek morbidite oranları ve ciddi mortalite ile Pakistan'da ciddi bir tehdit olarak ortaya çıkmıştır. Bu çalışmada, dört dang virüsü (DENV) serotipinden NS2B/NS3 proteazı, çeşitli tıbbi bitkilerden alınan 2350 fitokimyasal kullanılarak hedeflenmiştir.

Gereç ve Yöntemler: Fitokimyasallar, bağlanma alanına odaklanan AutoDock Vina kullanılarak NS2B/NS3 proteazlarına karşı kenetlenmeye tabi tutuldu ve etkin şekilde kenetlenmiş fitokimyasalları taramak için bağlanma enerjileri belirlendi. Farmakolojik özellikler ayrıca PreADMET

web sunucusu kullanılarak tüm fitokimyasallar için analiz edildi.
Bulgular: Bileşikleri taramak için bağlanma afiniteleri –4.0 ila –9.8 kcal/mol arasında ve eşik –9.0 kcal/mol olarak uygulanmıştır. Toplam 18 fitokimyasal bir ilacın tüm değerlendirme kriterlerini geçmesi için tarandı; 3'ü DENV1-NS2B/NS3 için, 5'i DENV2-NS2B/NS3 için, 6'sı DENV3-NS2B/NS3 için ve 4'ü DENV4-NS2B/NS3 içindi. *Erythrina variegate*'den Erycristagallin ve Osajin, *Fumaria indica*'dan PapraineA ve *Aloe vera*'dan Aloe-Emodin, sırasıyla –9.6 kcal/mol, –9.6 kcal/mol, –9.6 kcal/mol ve –9.2 kcal/mol, bağlanma afiniteleri ile DENV1, DENV2, DENV3 ve DENV4'ten NS2B/NS3 proteazının en güçlü inhibitörleridir.
Sonuç: Tüm 18 fitokimyasal maddenin etkin ilaç benzeri özellikleri, insanlardaki dang virüsü replikasyonuna karşı inhibisyon potansiyelini göstermektedir.

Anahtar Kelimeler: Dang virüsü; NS2B/NS3; Fitokimyasallar; Moleküler kenetlenme; ADMET; Sanal Tarama.

Introduction

Dengue fever is considered to be a very crucial disease with the diagnostic rate of infections of about 2 million people per year across the world. This fever is caused by the dengue virus, belonging to the *Flaviviridae* family. According to the World Health Organization, one-third of the world population is under the dengue virus epidemicity, with a variety of the strains of virus polypeptide [1, 2]. Five serotypes of dengue virus have been identified which are DENV-1, DENV-2, DENV-3 and DENV-4 and DENV-5 [3]. These first four serotypes are closely related in structure, however, they interact differently with the antibodies, present in the human blood serum. These viruses share a similarity of almost 65% in the genome while there are genetic variations within a single serotype. However, these four serotypes cause the same diseases despite having genomic variations. Genome of DENV-5, as compared to the other four serotypes, is less characterized and not much information is present regarding this serotype. The polypeptide of the dengue virus is encoded by a long single-stranded RNA, referred as positive sense RNA, and is further cleaved into ten different proteins (seven non-structural proteins and three structural) [4].

Membrane (M), Envelope (E) and Capsid (C) are the three structural proteins in dengue virus polypeptide whereas seven non-structural proteins are Non-structural protein 1 (NS1), Non-structural protein 2A (NS2A), Non-structural protein 2B (NS2B), Non-structural protein 3 (NS3), Non-structural protein 4A (NS4A), Non-structural protein 4B (NS4B) and Non-structural protein 5 (NS5) [5, 6]. The process of cleaving the viral precursor polyprotein into different functional proteins is carried out by the aid of viral and host cell proteases. NS3 protein constitutes N-terminal of the serine protease, which helps in cleaving the viral polypeptide into functional proteins. It is reported that NS3 protease has apo behavior and requires NS2B protein, a small protein of 14-kDa, as a cofactor which also stabilizes the NS2B/NS3 complex [7].

Serine proteases are observed to be the most studied proteases among all the four Serine, Aspartic, Metallo and Cysteine classes. The common catalytic apparatus which is Asp, His and Ser, plays a vital role in the conserved mechanism of catalysis among all the serine proteases [8]. The NS2B/NS3 serine protease has a heterodimeric behavior and it cleaves at various positions in the polypeptide of dengue virus such as capsid/membrane, NS2A/NS2B, NS2B/NS3, NS3/NS4A, NS4B/NS5, internal NS2A, NS3, and NS4A cleavage sites [9]. The viral replication is also sustained by this protease. All these functionalities of NS3 mark this protein as an important candidate for drug targeting by inhibiting its function. Various studies have been conducted for the sake of finding a putative and efficient drug candidate against NS2B/NS3 protease, using specific inhibitors and chemical libraries. The protease is reported to be the second largest protein of DENV genome and the conservancy of this protein among the four serotypes is 63–74% [10].

Dengvaxia (CYD-TDV) has been registered as a vaccine against dengue virus after passing through clinical trials. The vaccine is working effectively against seropositive individuals, however, the disease risk is higher in the individuals which are seronegative at the time of vaccination. The main drawback of the drug is the enhanced levels of antibodies, resulting non-protective dengue infection in seronegative patients [11].

It is observed that medicinal plants are being used for the treatment of various diseases since the ancient times. The plant extracts have chemically and biologically active compounds against various diseases. In the present study, NS2B/NS3 protease from four dengue virus serotypes has been targeted using 2350 phytochemicals from various medicinal plants locally present in the Indian Subcontinent. NS2B/NS3 protein of DENV-5 is not yet characterized therefore, it cannot be considered in the present study. Phytochemicals of these plants are used in this study for docking against DENV NS2B/NS3 and their interactions are observed for the discovery of effective drugs. These compounds are further evaluated on the basis of Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties. Experimental results show that 18

out of 2350 phytochemicals can be considered as effective drug-like antiviral agents against dengue fever in humans.

Material and methods

The present study targets the NS2B/NS3 protease from four dengue virus serotypes. The crystal structure of these proteases (PDB ID: 3L6P, 2FOM, 3U1I and 2VBC for dengue virus 1, 2, 3 and 4, respectively) were retrieved from RCSB databank [10, 12–14]. For exploring the potential inhibitors against these proteases, 2350 phytochemicals from various plants inhabited in India and Pakistan were searched through the literature survey and downloaded from PubChem. The phytochemical set comprised of 201 alkaloids, 387 terpenoids, 803 flavonoids, 204 isoflavonoids, 362 lignans, 112 isoflavones, 55 anthraquinones, and 226 miscellaneous phytochemicals [15, 16].

Molecular docking and binding energy calculation

The docking study was performed to analyse the inhibition role of phytochemicals against NS2B/NS3 proteases. The ligand and protein preparation was performed in AutoDock Tools while docking was performed using AutoDock Vina [17, 18]. Using the Discovery Studio 2.5 software, water molecules were removed and CHARMM27 force field was applied onto these protein structures. Subsequently, steric overlaps were removed and the structures were processed 1000 steps using the smart-minimizer algorithm [19]. Energy minimization and three-dimensional (3D) structure optimization was carried out for phytochemicals' structures using ACD ChemSketch [20]. The polar hydrogens were added in all the four receptors and the ligands (phytochemicals) using AutoDock Tools. The polar charges helped to enhance the process of interactions. Torsion adjustment was performed for the ligands as the ligand needs information regarding torsion. This illustrates which torsions are to be treated as rotatable during the docking. The 3D grid was designed to define the search space within receptors, focusing the binding pocket comprised of catalytic triad residues His, Asp and Ser (Table 1). The interactions of the phytochemicals were analyzed along with the estimation of binding energies using Autodock Vina [8]. Docking simulations were carried out with six different exhaustiveness heuristics i.e. E = 4, E = 8, E = 16, E = 32, E = 64 and E = 128. However, no improvement was observed in terms of binding after the exhaustiveness E = 8. The total number of dockings were 9400 (2350*4), performed by utilizing a JAVA based script to automate the docking process. The focused docking approach is reported to have higher accuracy as compared to the blind docking approach [6, 21].

Table 1: Grid box dimensions for receptors (Å^3).

Receptor	Grid box dimensions
DENV1-NS2B/NS3 (PDB ID: 3L6P)	22 × 22 × 30
DENV2-NS2B/NS3 (PDB ID: 2FOM)	18 × 18 × 20
DENV3-NS2B/NS3 (PDB ID: 3U1I)	16 × 18 × 18
DENV4-NS2B/NS3 (PDB ID: 2VBC)	20 × 18 × 18

ADMET and drug likeness prediction

Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) of all the compounds were calculated using PreADMET [22]. The program also helped in predicting the drug-likeness of these compounds. The predictions were made using the molecular structure file. Pharmacological properties and pharmacokinetics of the phytochemicals were analyzed using the physically significant descriptors and pharmaceutically relevant properties associated with the ligand molecules.

Results

Molecular docking and binding site prediction

All the four proteases were docked with the 2350 phytochemicals from various local medicinal plants. These phytochemicals interacted differently at the binding pocket, showing various binding affinities ranging from −4.0 to −9.8 kcal/mol. Among all the phytochemicals, 18 phytochemicals (three for DENV1-NS2B/NS3, five for DENV2-NS2B/NS3, six for DENV3-NS2B/NS3 and four for DENV4-NS2B/NS3) were selected on basis of effective drug-like properties and ADMET profiles (Table 2). The docking and binding energies of these selected 18 phytochemicals are discussed in this section.

Docking with DENV1-NS2B/NS3 protease

Oxysanguinarine, an alkaloid from *Fumaria indica*, docked strongly at the binding pocket of DENV1-NS2B/NS3 protease with Glu_{20}, Leu_{31}, Glu_{144}, Gln_{160} and Arg_{192} having the binding affinity −9.4 kcal/mol. Papracinine another alkaloid in

Table 2: Structures of screened phytochemicals.

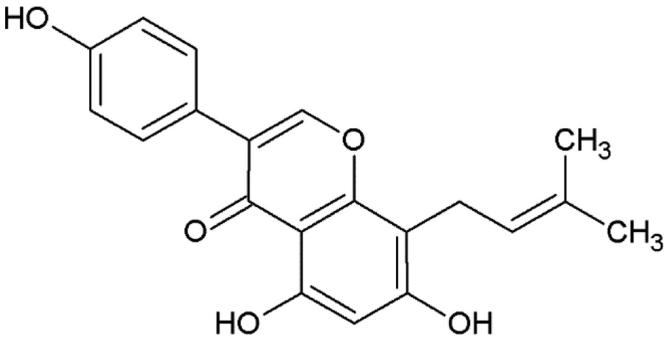
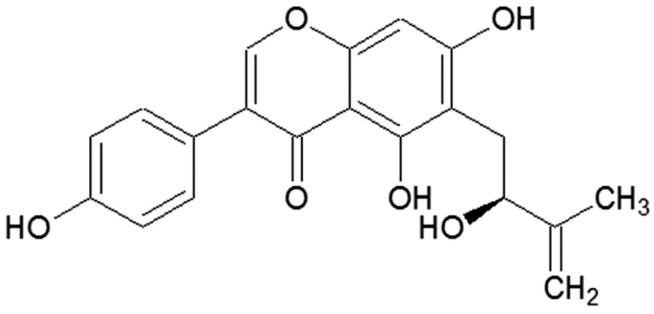
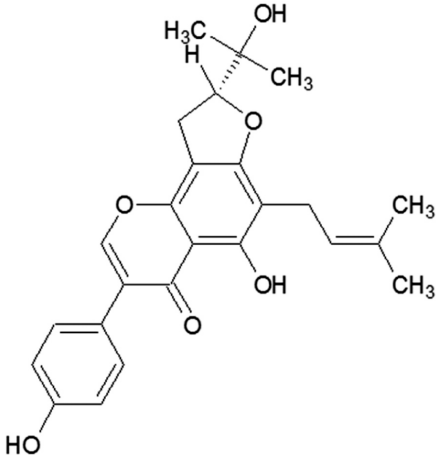
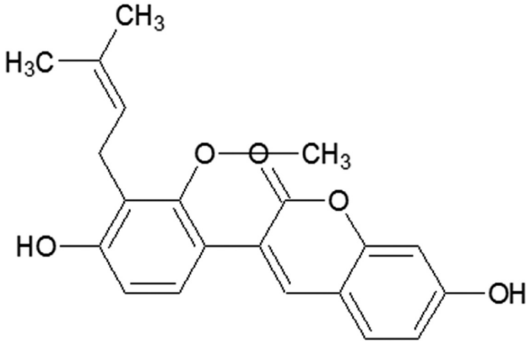
Phytochemicals	Structure
Aloe vera	
Aloe-Emodin	
Fumaria indica	
Oxysanguinarine	
Papracinine	
Fumaritine N-oxide	

Table 2 (continued)

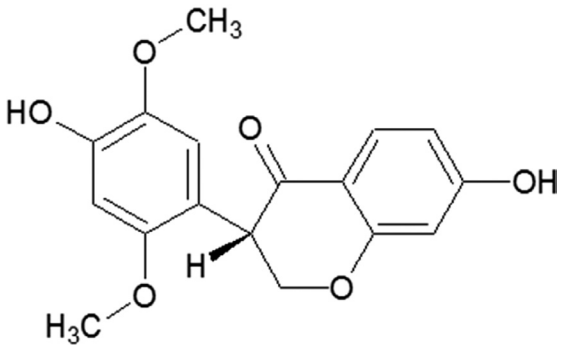
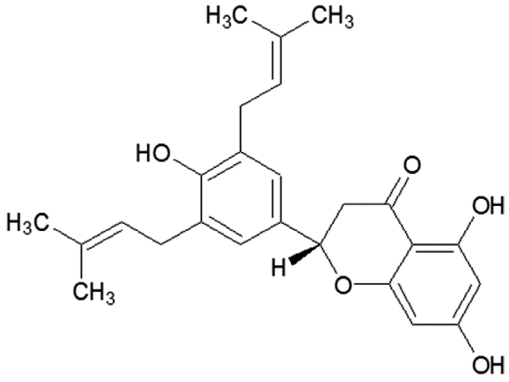
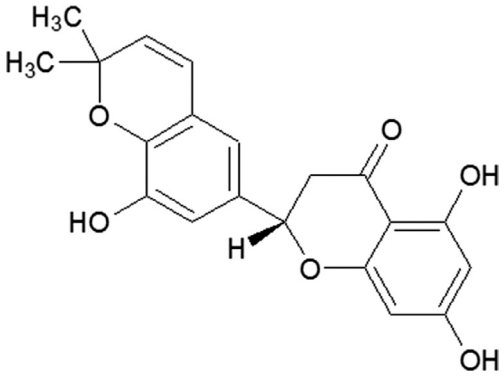
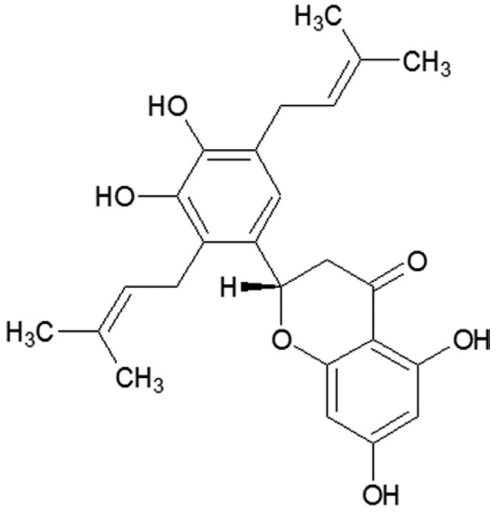
Phytochemicals	Structure
PapraineA	
PapraineB	
Erythrina variegate	
Erycristagallin	
Osajin	

Table 2 (continued)

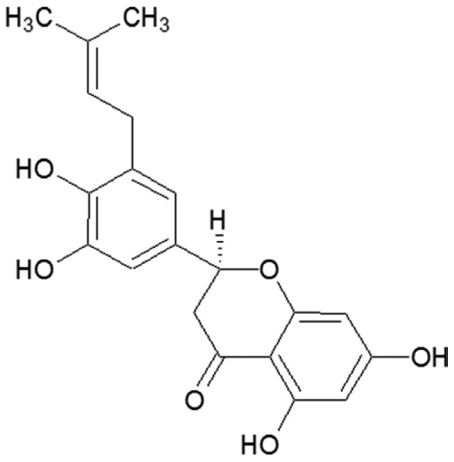
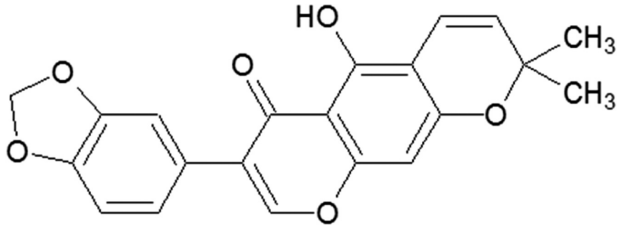
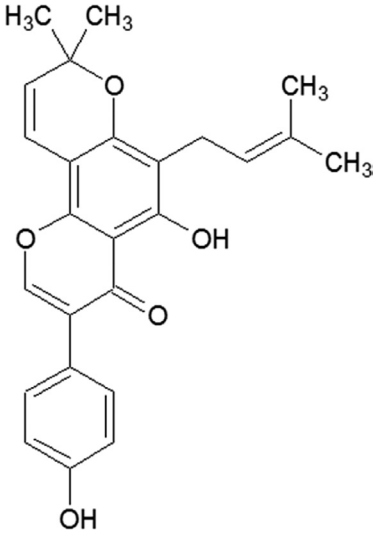
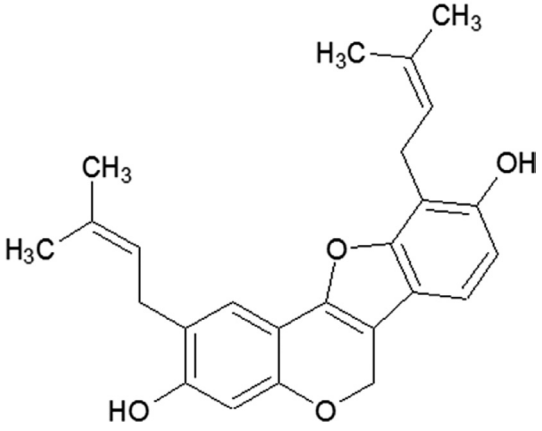
Phytochemicals	Structure
Robustone	
SigmodinB	
SigmodinA	
SigmodinC	

Table 2 (continued)

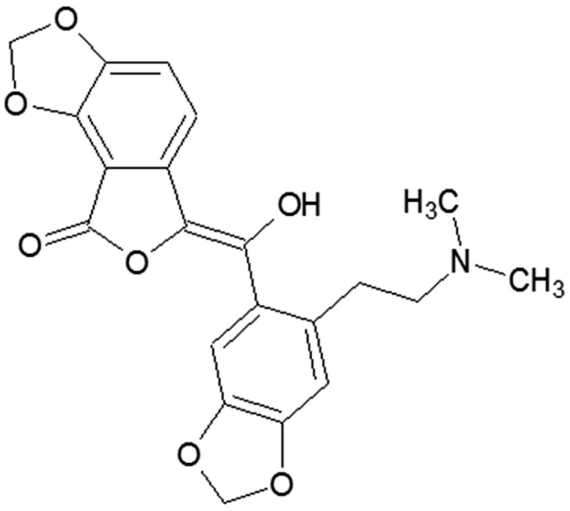
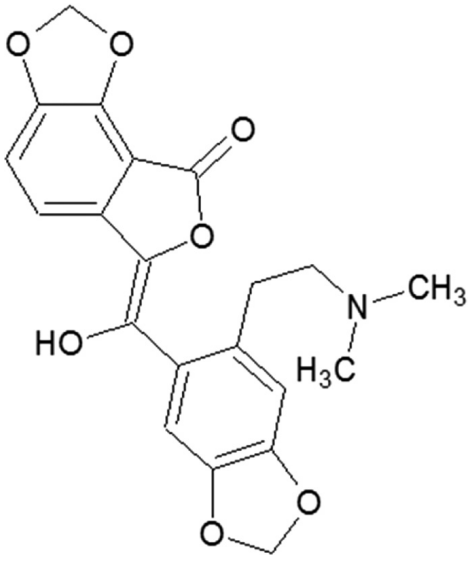
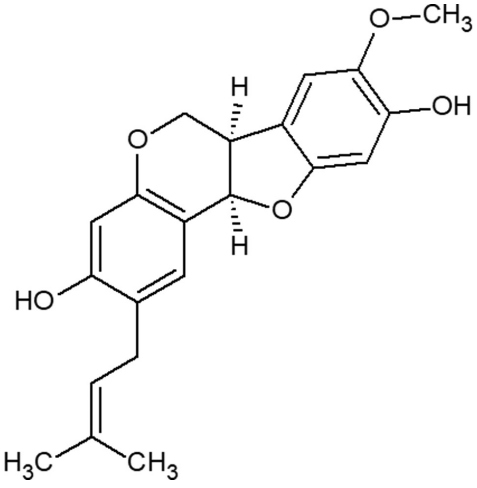
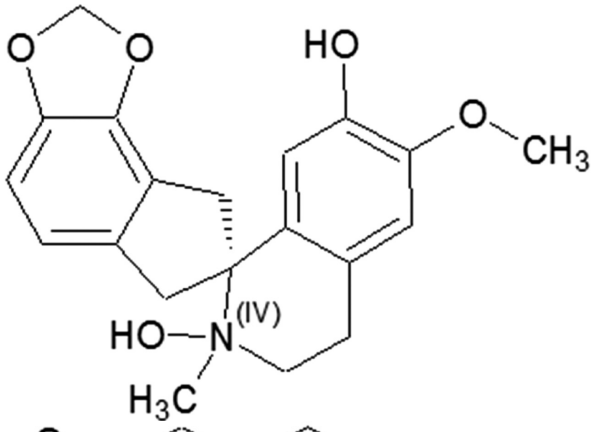
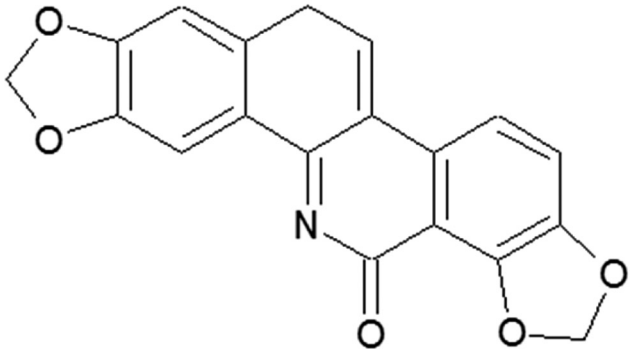
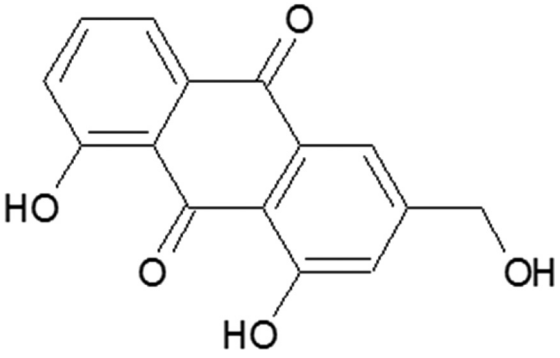
Phytochemicals	Structure
Abyssinone	
EryvarinA	
EryvarinB	

Table 2 (continued)

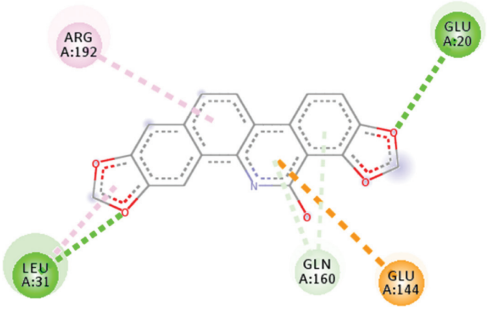
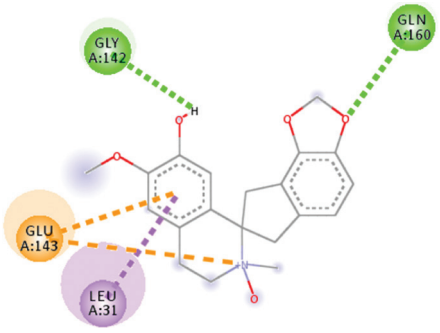
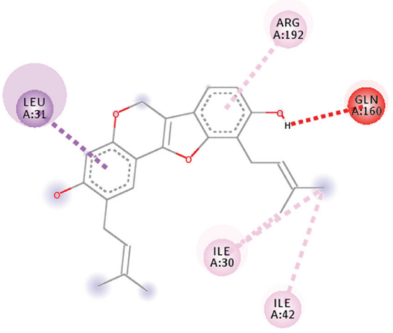
Phytochemicals	Structure
EuchrenoneB	
Laburnetin	
Lupiwighteone	

nature from the same plant, docked with the binding affinity of –9.2 kcal/mol. The residues involved in the interactions with Papracinine were Leu_{31}, Gly_{142}, Glu_{143} and Gln_{160}. Erycristagallin, a pterocarpan from *Erythrina variegate*, was observed to dock effectively with a binding affinity of –9.6 kcal/mol. The residues involved in these interactions were Ile_{30}, Leu_{31}, Ile_{42}, Gln_{160} and Arg_{192} (Table 3).

Docking with DENV2-NS2B/NS3 protease

Fumaritine N-oxide an alkaloid from *F. indica*, docked with the binding affinity –9.2 kcal/mol against DENV-2 serine protease at His_{51}, Arg_{54}, Val_{72}, Asp_{75} and Asn_{152}. Osajin, a flavonoid from *E. variegate* was docked with the protease, showing highest binding affinity –9.7 kcal/mol among all the phytochemicals. The residues involved in the interaction with the Osajin were Leu_{128}, Phe_{130}, Pro_{132}, Tyr_{150}, Gly_{151} and Gly_{153}. SigmodinA, another flavonoid from *E. variegate* docked with the protease at His_{51}, Leu_{128} and Pro_{132}, having a binding affinity of –9.0 kcal/mol. SigmodinB and SigmodinC, two flavonoids from *E. variegate* docked with the binding affinity of –9.4 kcal/mol. SigmodinB interacted at His_{51}, Asp_{75}, Leu_{128}, Pro_{132} and Val_{154} and SigmodinC involved His_{51}, Asp_{75}, Leu_{128}, Pro_{132}, Ser_{135} andGly_{153} of the protease (Table 4).

Table 3: Docking results for DENV1-NS2B/NS3.

Phytochemicals	Binding sites (DENV1-NS2B/NS3)	Binding affinities (kcal/mol)
Fumaria indica		
Oxysanguinarine		−9.4
Papracinine		−9.2
Erythrina variegate		
Erycristagallin		−9.6

Docking with DENV3-NS2B/NS3 protease

PapraineA and PapraineB, two alkaloids from *F. indica* docked with binding affinity −9.6 kcal/mol. PapraineA interacted with His_{51}, Ser_{135} and Tyr_{161} and PapraineB interacted with His_{51}, Asp_{81}, Gly_{133} and Gly_{151}at the binding site of this protease. The results identified that PaprarineA and PapraineB are potential inhibitors of the DENV3-NS2B/NS3 protease. Robustone from *E. variegate*, being isoflavone in nature, interacted with Val_{36}, His_{51}, Thr_{83}, Mer_{84} and Pro_{132} with the binding affinity determined was −9.0 kcal/mol. Abyssinone from *E. variegate*, a flavonoid, was docked with the protease having a binding affinity of −9.4 kcal/mol and established interactions with His_{51}, Thr_{83}, Mer_{84}, Tyr_{150} and Tyr_{161} residues of the binding site. EryvarinA and EryvarinB, two flavonoids from *E. variegate* docked with the binding affinities of −9.0 and −9.3 kcal/mol, respectively. The EryvarinA was docked atHis_{51}, Asp_{75}, Asp_{81}, Pro_{132} and Gly_{151} whereas EryvarinB docked at His_{51}, Gly_{133}, Tyr_{150}, Gly_{153} and Tyr_{161} (Table 5).

Docking with DENV4-NS2B/NS3 protease

Aloe-Emodin, an anthraquinone from the plant *Aloe vera*, docked with His_{51}, Asp_{75} and Asn_{152} of the binding

Table 4: Docking results for DENV2-NS2B/NS3.

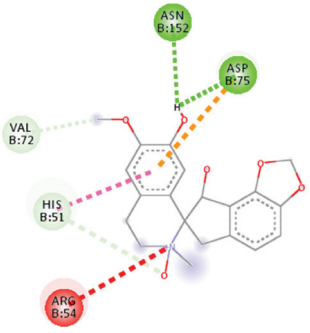
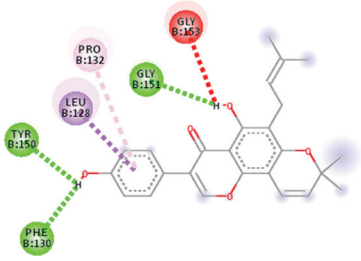
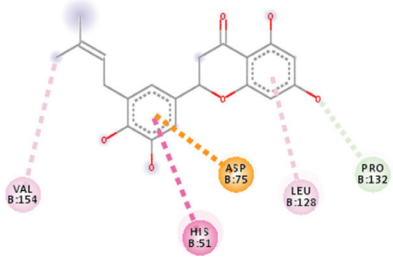
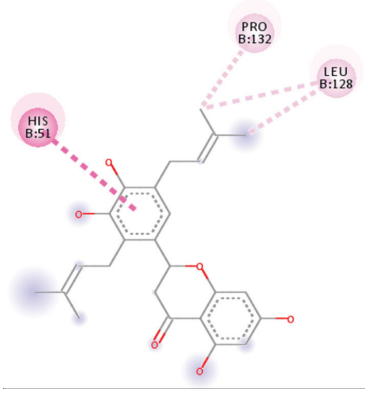
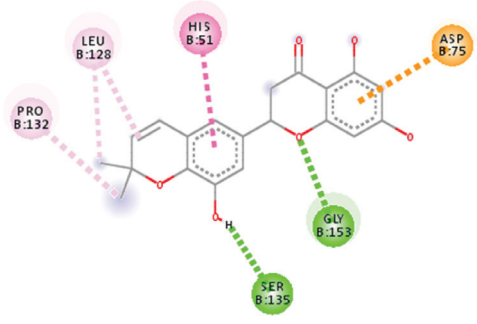
Phytochemicals	Binding sites (DENV2-NS2B/NS3)	Binding affinities (kcal/mol)
Fumaria indica		
Fumaritine N-oxide		−9.2
Erythrina variegate		
Osajin		−9.7
SigmodinB		−9.4
SigmodinA		−9.0
SigmodinC		−9.4

Table 5: Docking results for DENV3-NS2B/NS3.

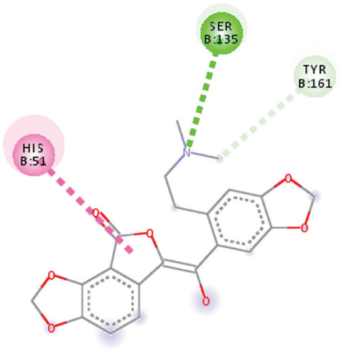
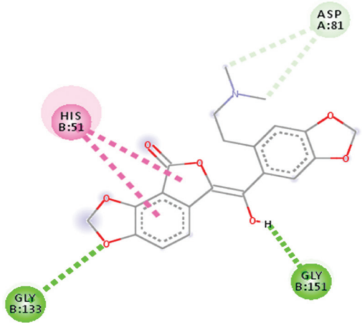
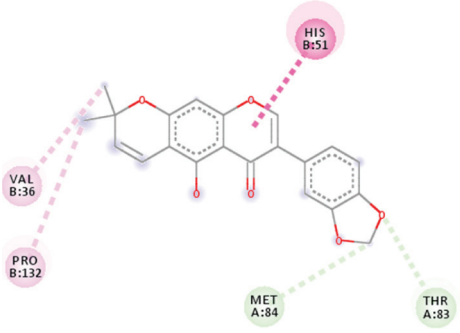
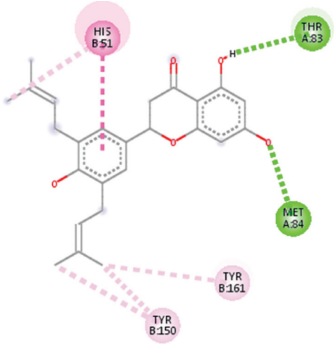
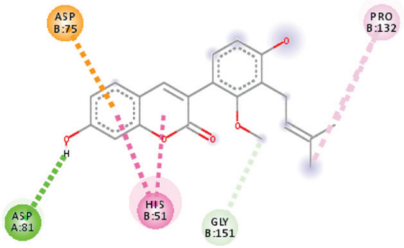
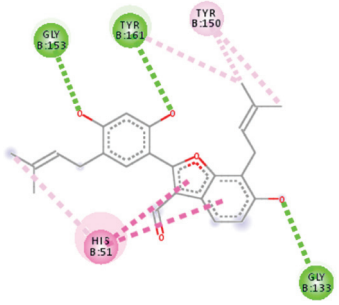
Phytochemicals	Binding sites (DENV3-NS2B/NS3)	Binding affinities (kcal/mol)
Fumaria indica		
PapraineA		−9.6
PapraineB		−9.6
Erythrina variegate		
Robustone		−9.0
Abyssinone		−9.4

Table 5 (continued)

Phytochemicals	Binding sites (DENV3-NS2B/NS3)	Binding affinities (kcal/mol)
EryvarinA		−9.0
EryvarinB		−9.3

pocket of DENV4-NS2B/NS3, having a binding affinity of −9.2 kcal/mol. EuchrenoneB, a flavonoid from *E. variegate* docked with the binding affinity of −9.0 kcal/mol with Arg_{54} and Pro_{132} at the binding site of the protein. Laburnetin and Lupiwighteone, two isoflavones from *E. variegate*, both docked with the binding affinity of −9.1 kcal/mol making interactions withAsn_{152}, $Ala_{164}$and Thr_{166}, and Trp_{50}, His_{51}, Arg_{73} respectively (Table 6).

ADMET and drug likeness prediction

All the successfully docked phytochemicals having the binding affinity −9.0 kcal/mol and above were evaluated for their drug-likeness and ADMET profiles. One of the most effective and important factor to be considered in ADME profiles is the Lipinski's rules non-violations. Physical descriptors such as molecular weight, the hydrogen donor-acceptor bonds and the lipophilicity (log*P*) of the chemicals are evaluated in these rules. A total of 98 phytochemicals obeyed Lipinski's rules and showed drug like behavior. The Rodent and Ames tests for toxicity prediction helped in further evaluation of these compounds. The results showed that out of these 98 phytochemicals, 53 were non-carcinogenic, non-mutagenic and non-toxic.

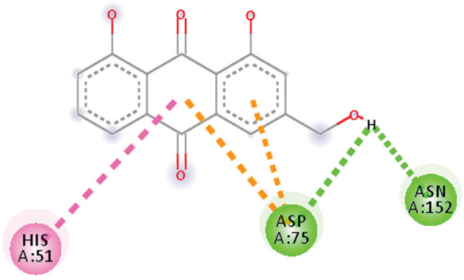
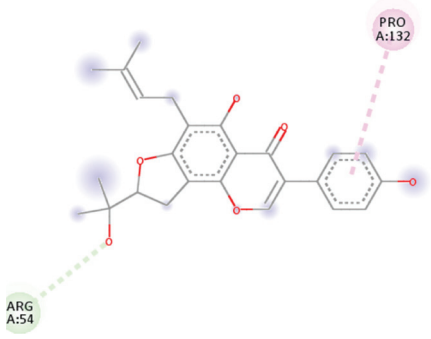
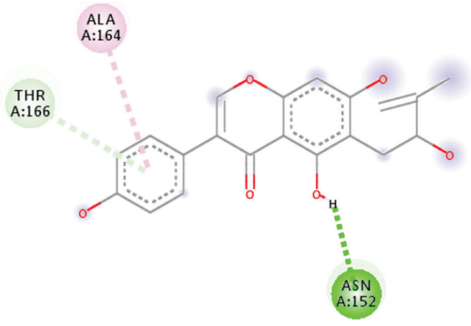
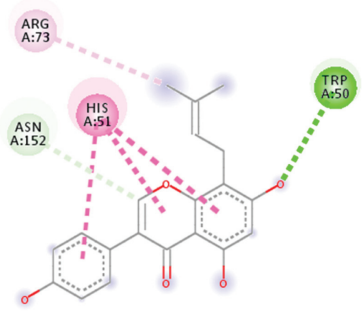
These compounds were further evaluated on the basis of blood brain barrier (BBB) penetration behavior. From the 53 compounds, 18 non-penetrating compounds were observed to be passing BBB evaluation of which six were from *F. indica*, 12 from *E. variegate* and one was from *A. vera* (Table 7). These compounds can be used as potential drugs, having strong inhibitory properties for NS2B/NS3 protein from dengue virus.

Estimated solubility (ESOL) of the phytochemicals from two plants (*A. vera* and *F. indica*) was observed to be high as compared to that of the phytochemicals from *E. variegate*. Erycristagallin, Osajin, SigmodinA, Abyssinone and EryvarinB from *E. variegate* showed poor solubility. Gastrointestinal Absorption of all the selected 18 phytochemicals was also high which reflected the effectiveness of these chemicals to be used as drugs.

Discussion

The dengue fever is a pathogenic disease caused by the dengue virus communicated through an insect vector. Virus replication in host cells is carried out by the non-structural proteins. These non-structural proteins are also important for carrying out some other functionalities in host cells. In dengue virus, NS2B/NS3 protease is a trypsin-like serine protease, which plays an important role in cleavage of the polypeptide, transcribed from the viral genome. It is also known to play an important part in viral replication. Due to its multi-functionality, the protease has

Table 6: Docking results for DENV4-NS2B/NS3.

Phytochemicals	Binding sites (DENV4-NS2B/NS3)	Binding affinities (kcal/mol)
Aloe vera		
Aloe-Emodin		−9.2
Erythrina variegate		
EuchrenoneB		−9.0
Laburnetin		−9.1
Lupiwighteone		−9.1

always been a target of drug discovery in order to cure the dengue fever. The present study focuses on screening and development of inhibitors of the targeted protease. A total of 2350 phytochemicals from various medicinal plants were docked against the protease. Many studies have been reported focusing the in vitro and in vivo analysis for the development of drugs against this protease. It is always preferred to perform an in silico analysis before conducting any experimental work, as this kind of analysis is cheap in terms of cost and time consumption.

Triterpenoids phytochemicals from *Azadirachta indica* were evaluated by Dwivedi et al. against NS2B/NS3

Table 7: Pharmacological properties and drug likeness of phytochemicals.

Phytochemicals	Estimated solubility (ESOL)	ESOL class	Gastrointestinal (GI) absorption	Blood-brain barrier (BBB) permeant	Lipinski's rules violations	Rodent test	Ames test	Carcinogenic
Aloe vera								
Aloe-Emodin	−3.04	Soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
Fumaria indica								
Oxysanguinarine	−2.95	Soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
Papracinine	−3.95	Soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
Fumaritine N-oxide	−3.34	Soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
PapraineA	−4.13	Moderately soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
PapraineB	−4.13	Moderately soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
Erythrina variegate								
Erycristagallin	−6.58	Poorly soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
Osajin	−6.24	Poorly soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
Robustone	−5.06	Moderately soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
SigmodinB	−4.68	Moderately soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
SigmodinA	−6.22	Poorly soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
SigmodinC	−4.54	Moderately soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
Abyssinone	−6.35	Poorly soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
EryvarinA	−5.2	Moderately soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
EryvarinB	−6.27	Poorly soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
EuchrenoneB	−5.7	Moderately soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
Laburnetin	−4.46	Moderately soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic
Lupiwighteone	−5.11	Moderately soluble	High	No	0	Non toxic	Non toxic	Non carcinogenic

protease and the results of molecular docking showed that nimbin, desacetylnimbin and desacetylsalannin inhibit the NS2B/NS3 protease with the binding affinity −5.56, −5.24 and −3.43 kcal/mol, respectively. These phytochemicals made interactions with Val_{36}, Arg_{73}, Pro_{132}, Gly_{133}, Gly_{153} and Val_{154}, similar to the results of the present study [23]. Yong et al. targeted the serine protease from dengue virus 3 with the compounds from *Murraya koenigii*. MSC1308 from *M. koenigii* was observed to be a potent inhibitor, making interactions with the Met_{84}, Asp_{129}, Phe_{130}, Tyr_{150}, Asn_{152}, Gly_{153}, Val_{154}, Val_{155}, and Tyr_{161} residues with the binding affinity −7.8 kcal/mol [24]. Similarly, in the present study, SigmodinA from *E. variegate* was docked with the protease, showing highest binding affinity −7.8 kcal/mol among all the phytochemicals while making interactions with His_{23}, Ile_{30}, Leu_{31}, Ile_{42}, Gln_{160} and Arg_{192} at the binding pocket. Wu et al. proposed some novel inhibitors against NS2B/NS3 protease from dengue virus 2 and 3 from the diaryl (thio) ethers class. The interactions of these protease inhibitors were observed at catalytic triad residues His_{51}, Asp_{75}, and Ser_{135} of the active site of the protease [25]. In another study, 20 ligands from PubChem/Drug bank were docked against the serine protease from dengue virus 2 which were bound with the various surrounding residues at the binding site [26].

The screened 18 phytochemicals belonged to the three different medicinal plants i.e. five from *F. indica*, 12 from *E. variegate* and one from *A. vera*, all of are found in Pakistan. *Aloe vera* has been used for a long period of time for health, beauty, and skin care purposes, and has potentially active compounds such as amino acids, enzymes, lignin, minerals, salicylic acids, saponins, sugars and vitamins [27]. *Fumaria indica* is an annual herb and is recommended for the treatment of blood disorders, chronic skin diseases, cough, fevers, and urinary diseases [28]. *Erythrina variegate* commonly known as a coral tree and is cultivated due to its ornamental, medicinal, and nitrogen-fixing properties [29]. The phytochemicals from this plant have been used for anti-asthmatic, bacterial infection, cough, febrifuge, fever, helminthiasis, insomnia, inflammation, nervine sedative, cuts and wounds [30]. ADMET of the 18 selected phytochemicals revealed that these compounds have very effective drug like properties along with the non-toxic, non-carcinogenic and non-mutagenic nature. The ADMET was considered as final criteria of screening a huge number of phytochemicals, following various reported techniques [6, 31–33].

Computational approaches for drug discovery and development are proven to be effective and time efficient as they are not based on difficult laborious works. The protein-ligand docking elucidates the mechanism of inhibition along with the specificity and efficiency of that ligand as an inhibitor. ADMET evaluation helps in computational determination of absorption, distribution, metabolism, excretion and toxicity for use in humans. In silico studies help in identifying selective compounds efficiently and further these compounds can be considered for in vitro analysis because in vitro analysis of a large number of compounds is a time consuming and costly process. It is common practice adopted by researchers to perform computational analysis of compounds for their efficacies against enzymes before going to perform in vitro or in vivo investigations.

The association of drug candidate (ligand) to its target receptor is a fundamental binding reaction and the aim of the computer-aided drug discovery is to find small molecules having strong inhibitory or activating action against the biological targets. The strength of inhibition or activation is elucidated through binding affinity [34]. In the present study, the screened 18 phytochemicals showed high inhibitory potential against the NS2B/NS3 proteases as binding affinities of these compounds is ≥−9.0 kcal/mol. In a previous study, the inhibitory effect of baicalein and baicalin against DENV Envelope, NS2B-NS3 protease and NS5 protein has been identified using molecular docking approaches by Hassandarvish et al. [35]. Both compounds have shown binding affinities −8.0 kcal/mol and −7.5 kcal/mol, respectively against NS2B-NS3 protease. In vitro analysis revealed that IC_{50} was 68.65 μg mL^{-1} for baicalein and 144 μg mL^{-1} for baicalin against NS2B-NS3 protease, elucidating the potential of these two compounds against DENV replication [35]. It is manifested that the screened 18 compounds, in our study, can effectively inhibit in vitro intracellular replication of DENV because these compounds have higher binding affinities (≥−9.0 kcal/mol) as compared to that of baicalein and baicalin. Makau et al. identified potential inhibitors using in silico and in vitro studies against nucleoprotein from Influenza A virus. A set of 9430 compounds was docked against the protein and the screened compound (NUD-1), belonging to 4-hydroxyquinolinone class, was considered for further in vitro analysis. In vitro analysis revealed the potential of NUD-1 against Influenza A virus as the inhibitory concentration ranged within 1.8–2.1 μM for NUD-1 [36].

The proposed study focused on identification of potential inhibitors against NS2B/NS3 protease from dengue virus to control the viral replication. After the evaluation of the results, 18 phytochemicals are identified as potent inhibitors against dengue fever. The phytochemicals showed strong inhibition against NS2B/NS3 proteases from four serotypes of DENV. Pharmacological assessment of the compounds revealed the effective drug-likeness and

pharmacokinetics of these phytochemicals to be used against dengue fever and other crucial diseases caused by DENV. Therefore, the effect of these inhibitors can be further elucidated through in vitro and in vivo analysis in the cessation of intracellular replication of dengue virus, prior to the use as drugs in humans.

Conflict of interest statement: The authors declare no conflict of interest.

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