Research Article

In Silico Elucidation of Potent Inhibitors from Natural Products for Nonstructural Proteins of Dengue Virus

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Medicinal plants have been used from the beginning of human civilization against various health complications. Dengue virus (DENV) has emerged as one of the most widespread viruses in tropical and subtropical countries. Yet no clinically approved antiviral drug is available to combat DENV infection. Consequently, the search for novel antidengue agents from medicinal plants has assumed more insistence than in previous days. This study has focused on 31 potential antidengue molecules from secondary metabolites to examine their inhibitory activity against DENV nonstructural proteins through molecular docking and pharmacokinetics studies. In this research, the wet lab experiments were tested on a computational platform. Agathisflavone and pectolinarin are the top-scored inhibitors of DENV NS2B/NS3 protease and NS5 polymerase, respectively. Epigallocatechin gallate, Pinostrobin, Panduratin A, and Pectolinarin could be potential lead compounds against NS2B/NS3 protease, while acacetin-7-O-rutinoside against NS5 polymerase. Moreover, agathisflavone ($LD_{50} = 1430$ mg/kg) and pectolinarin ($LD_{50} = 5000$ mg/kg) exhibited less toxicity than nelfinavir ($LD_{50} = 600$ mg/kg) and balapiravir ($LD_{50} = 824$ mg/kg), and the reference drugs. Further research on clinical trials is required to analyze the therapeutic efficacy of these metabolites to develop new potential drug candidates against different serotypes of DENV.

1. Introduction

Dengue, a neglected tropical disease, is a growing threat to public health, especially in developing countries, and is the most common arboviral infection in the world [1]. Dengue was listed in the top ten global health threats of 2019 by the World Health Organization (WHO), which puts nearly 40% of the worldwide population at risk of dengue infection, and about 390 million cases are reported per year [2]. Mosquito-borne dengue cases are rapidly increasing in different tropical and subtropical regions, even in the more expanded form [3–5]. WHO has planned to reduce mortality and morbidity caused by the dengue virus (DENV) by 50% and 25%, respectively, by 2020 [6]. DENV is an arthropod-borne flavivirus having antigenically distinct serotypes 1–4 (DENV-1, DENV-2, DENV-3, and DENV-4) [7, 8]. These serotypes vary from one another in amino acid sequences by 25 to 40%, and genotypes by approximately 3% [9]. However, the new serotype of DENV (DENV-5) was also isolated in 2013 and could emerge due to natural selection and genetic recombination [10].

DENV is an enveloped, single-positive-stranded RNA virus of ~11 kilobases with a single open reading frame belonging to the *Flaviviridae* family [11]. DENV is closely related to the West Nile virus, yellow fever virus, hepatitis C virus, and encephalitis viruses [11, 12]. The molecular basis

of DENV virulence remains unclear since a definitive receptor for the entry of the virus has not been recognized. The *in vivo* study suggests that DENV targets include macrophages, dendritic cells, monocytes, mast cells, and probably endothelial and hepatocytes [9]. Rapid urbanization, accelerated population growth, global warming, inefficient mosquito management, and a lack of health care facilities are the major reasons behind the increased cases of DENV infections [13].

Dengue fever, dengue hemorrhagic fever, and dengue shock syndrome are the health threats developed by DENV infection [14]. Fever, frontal headache, rash, swollen glands, nausea, vomiting, nasal stuffiness, sore throat, retro-orbital pain, abdominal, muscle, and joint pain are the most common symptoms that arise after evading DENV on human dendritic cells through vector mosquitoes *Aedes aegypti* and *Aedes albopictus* [15].

The genome of DENV encodes structural proteins; core or capsid (C), premembrane (prM or M), envelope (E), and nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) required for the viral cycle [16, 17]. Nonstructural proteins are involved in viral RNA replication and evasion of host immunity, while receptor binding, fusion, maturation, and assembly of DENV are controlled by structural proteins [11, 18]. These proteins could be a target to prevent DENV infections. Due to variations in the genetic material of DENV, the development of the curative method is difficult [19, 20].

Currently available vaccines or drugs are not very satisfactory against DENV and its complications, so the development of sound, practical, and long-lasting therapeutic approaches is on the verge of controlling and preventing fatal outcomes [21, 22]. Genetically engineered mosquitoes, engineered antibodies, and the release of insect-dominant lethal and sterile insect techniques are the latest strategies to control DENV outbreaks [23]. Various *in vitro* and *in vivo* studies worldwide showed different antiviral compounds (Figure 1) from natural products inhibit protease and replication proteins of DENV (Table 1S). These compounds are further analyzed to develop them as possible antidengue drugs. Artificial intelligence (AI) has drawn a lot of attention in drug design, screening potential drug candidates, and quantitatively studying their structure-activity affinity [24, 25]. Here, we carried out molecular docking and pharmacokinetics studies of the antidengue compounds that aid drug discovery programs.

2. Methods

2.1. Selection and Preparation of Proteins and Ligands.
NS2B/NS3 Protease (PDB ID: 2FOM, Resolution - 1.50 Å) [26] and NS5 Polymerase (PDB ID: 2J7U, 1.85 Å) [27] of DENV were selected as targets (Tables 2S and 3S). The open conformation of NS2B/NS3 protease is favored over the closed conformation to study ligand binding and conformation change [28]. The crystal structures of these target proteins were retrieved from the Protein Data Bank (PDB) (https://www.rscb.org/). The energy was minimized, hydrogens were added; water and ligand molecules were eliminated from protein structures using the Molecular operating environment (MOE) program [29, 30]. This refined structure was then used as a receptor for docking analyses.

2.2. Preparation of Ligands.
Compounds with antidengue activities isolated from natural products were selected as ligands through a literature survey (Table 1S). Experimented molecules through *in vitro* or *in vivo* studies were examined in the computational platform to know their therapeutic potential. The chemical structures of active antidengue compounds as ligands were constructed using ChemDraw (Cambridge Soft) and processed or optimized for docking using the MOE program.

2.3. Identification of the Docking Site.
The binding sites for NS2B/NS3 protease and NS5 polymerase were identified by using the Site-Finder module of MOE Software [30] (Table 4S) and cross-checked with the available literature [26, 27, 31–35]. After finding active sites, a grid box was created on receptors (target proteins). Blind docking was performed using nelfinavir and balapiravir as standard drugs for NS2B/NS3 protease and NS5 polymerase, respectively.

2.4. Physicochemical Properties and Secondary Structure of Proteins.
The physicochemical properties of the dengue proteins (PDB ID: 2J7U and 2FOM) were accessed using the Expasy ProtParam web server [36, 37], which helps to determine the essential characteristics of a protein in different biological forms. The described server was used to calculate molecular weight, amino acid composition, molecular formula, theoretical isoelectric point (Pi), the total number of positive and negative residues ($+R/-R$), extinction coefficient (EC), instability index (AI), aliphatic index, and grand average of hydropathicity (GRAVY).

Secondary structure prediction is an *in silico* approach that assigns all residues from all conceivable states based solely on hydrogen bonding patterns and some geometric constraints [38]. The annotated secondary structure elements (SSEs) were predicted using the self-optimized prediction method with alignment (SOPMA) [39].

2.5. Molecular Docking Analysis.
GOLD (Genetic Optimization for ligand Docking) version 4.0.1 based on a genetic algorithm with MOE version 3.12 was used to examine the binding of the ligands to the target proteins 2FOM and 2J7U of DENV. The ligand interactions, including 2D and 3D representations and distance between ligand and receptor protein interactions, were studied by the ligand interaction module in the MOE Program and visualized on the Biovia Discovery Studio Visualizer for graphical analysis and final processing.

GOLD fitness is a force field-based scoring function exploring binding modes of ligands with receptor proteins containing four terms: (a) protein-ligand hydrogen bond energy (external H-bond); (b) protein-ligand van der Waals
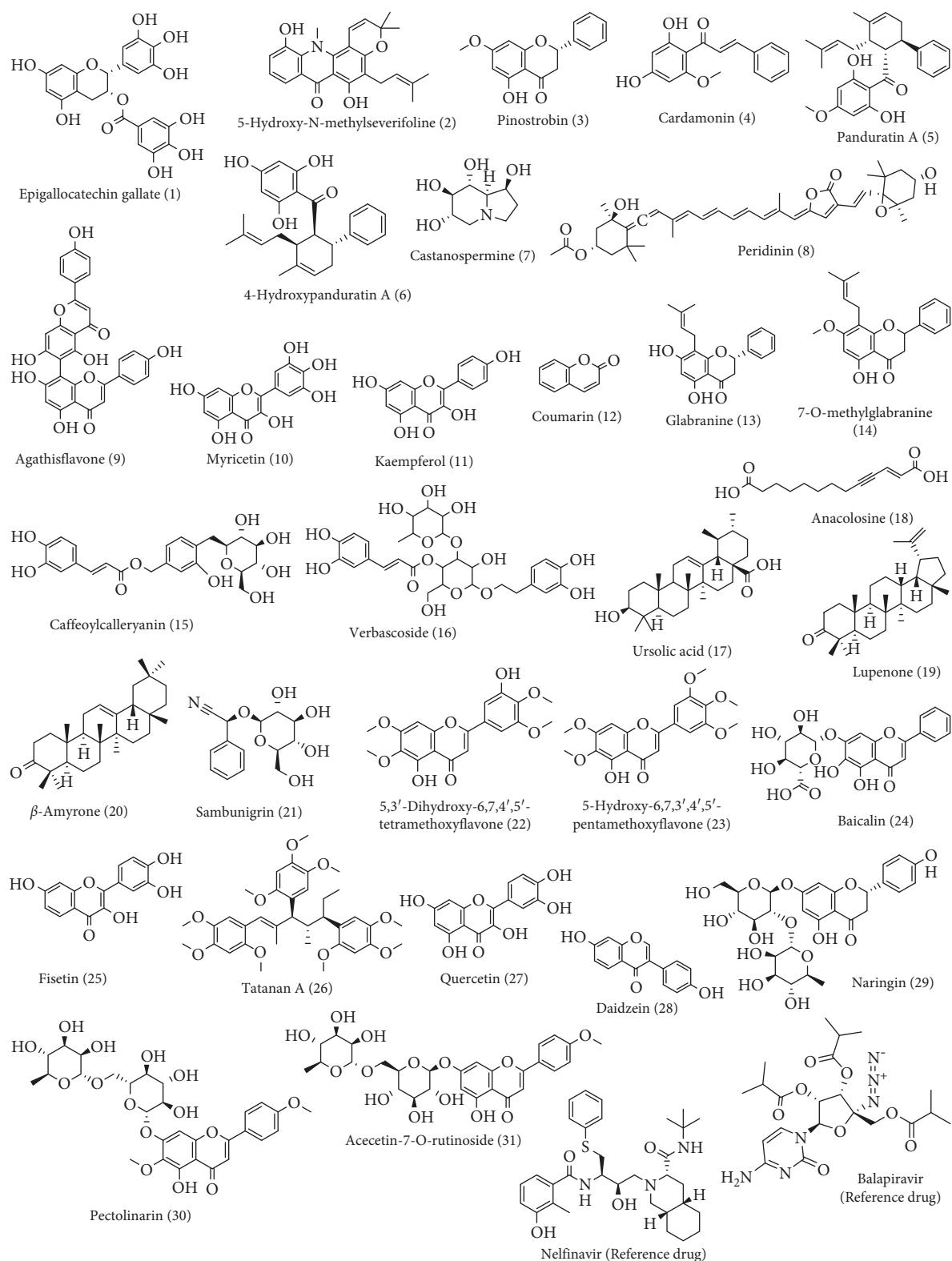
Figure 1: Antidengue compounds isolated from various medicinal plants.

energy (external vdw); (c) ligand internal van der Waals energy (internal vdw); and (d) ligand intramolecular hydrogen bond energy (internal H-bond) [40].

$$\text{GOLD fitness} = S_{\text{hb\_ext}} + S_{\text{vdw\_ext}} + S_{\text{hb\_int}} + S_{\text{vdw\_int}}, \quad (1)$$

where $S_{\text{hb\_ext}}$ = protein-ligand hydrogen-bond score; $S_{\text{vdw\_ext}}$ = protein-ligand van der Waals score; $S_{\text{hb\_int}}$ = contribution due to intramolecular hydrogen bonds in the ligand; and $S_{\text{vdw\_int}}$ = contribution due to intramolecular strain in the ligand.

The GOLD fitness score was taken as a basis to rank the binding modes of the ligand. A GOLD fitness score was obtained by subtracting intramolecular terms from GOLD fitness [40]. Higher the GOLD fitness score of ligands, higher the putative inhibitors of receptor proteins.

2.6. Estimation of Binding Energy Using a Semiempirical Method. To estimate binding energy, we applied a semiempirical method in-built to AutoDock Vina [41]. The semiempirical prediction of total binding energies is considered well suited for the relative rankings [42]. The pIC_{50} value was calculated using the formula: $pIC_{50} = -log(IC_{50}^* 10^{-9})$ and $\Delta G_{Experimental}$ was calculated by the equation: $\Delta G_{Exp.} = -RT \ln (_pIC_{50})$ [43].

2.7. Validation of Molecular Docking. The docking results were validated by extracting the standard nelfinavir and balapiravir from their original binding site and redocking them into the same positions. The lowest energy pose obtained on redocking and the previous docking position of compounds were superimposed, and its root mean square deviation (RMSD) was calculated. To validate the docking process, the RMSD must be within the reliable range of 2 Å [44, 45].

2.8. Prediction of Pharmacokinetic Parameters. Pharmacokinetic properties such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) of selected potent antidengue compounds were predicted by using chemoinformatic tools (pkCSM and ProTox-II) [46, 47]. *In silico* structure-based toxicity analysis by ProTox-II gives information about different levels of toxicity such as oral toxicity, organ toxicity, toxicological endpoints (carcinogenicity, cytotoxicity, hepatotoxicity, immunotoxicity, and mutagenicity), toxicological pathways, and active targets with a confidence score [46].

3. Results

3.1. Physicochemical Properties and Secondary Structure of Proteins. Expasy ProtParam allows the computation of various physical and chemical parameters for a given protein. The physicochemical properties of dengue proteins are depicted in Table 1, which displayed that the selected proteins (2J7U and chain B of 2FOM) are stable, which is given by the instability index.

Secondary structure elements of a protein like an alpha helix, beta-strands, and turns are the key constituent parts of protein structure which are displayed in Table 2 and the graphical representation is shown in Figure 1S.

3.2. Molecular Docking Analysis. GOLD fitness scores, hydrogen-bonding interactions between targets and secondary metabolites, interaction type, and bond length of the docking were shown in Tables 3, 4, 5S, and 6S. The 2D and 3D interactions of the top-scored metabolites and commercial drugs with the target proteins were shown in Figure 2 and Supplementary Figures 2S–7S. The details of the molecular

properties of standard drugs and secondary metabolites are shown in Supplementary Table 7S. Supplementary Tables 8S and 9S illustrate the theoretical (calculated) and experimental binding energies of secondary metabolites.

3.2.1. Molecular Docking Analysis of NS2B/NS3 Protease. The interactions mode obtained by molecular docking for Agathisflavone (9) and the standard drug nelfinavir were illustrated in Figure 2 and Supplementary Figure 2S. The docking of ligands was evaluated based on their GOLD fitness score and lowest binding energy values (kcal/mol). Agathisflavone (9) (GOLD fitness score 64.12, $\Delta G = -8.6$ kcal/mol) showed hydrogen bonds with the amino acids Thr 149, Asn 196, and Val 175 with bond distances of 3.4 Å, 3.4 Å, and 2.1 Å, respectively. Furthermore, nelfinavir showed H-bonding residue with Trp 118 at a bond distance of 1.4 Å. Additionally, Epigallocatechin gallate (1), Pinostrobin (3), Panduratin A (5), Peridinin (8), and Pectolinarin (30) were among the top-scored compounds with NS2B/NS3 Protease (Table 3).

3.2.2. Molecular Docking Analysis for NS5-Polymerase. In *silico* studies showed pectolinarin (30) as a top-scored inhibitor of NS5-polymerase with a GOLD fitness score of 50.60 ($\Delta G = -7.8$ kcal/mol) and interacted with Glu 197, Ile 487, and Arg 482 amino acids with bond distances of 2.0/3.3 Å, 3.8 Å, 1.7/2.6/2.6/2.4 Å, respectively (Figure 2). It showed Π-cation interactions with Lys122 and Lys123. Acacetin-7-O-rutinoside (31) shows H-bonding interaction with Lys123, Thr126, Asn127, and Arg482 with a GOLD fitness score of 46.10 (Table 4). Similarly, balapiravir was also found to interact with Arg482 and Asn127 at bond lengths of 1.6/2.3 Å and 2.0 Å, respectively (Figure 6S).

3.3. Estimation of Binding Energy. The relationship between theoretical and experimental binding free energies, IC50, and pIC50 of secondary metabolites for NS2B/NS3 Protease and NS5-polymerase was studied (Tables 8S and 9S). The theoretical and experimental binding free energies showed a good correlation. The binding free energies of top-scored inhibitors; epigallocatechin gallate (1), pinostrobin (3), panduratin A (5), agathisflavone (9), pectolinarin (30), and acacetin-7-O-rutinoside (31) are −7.7, −7.7, −7.9, −8.6, −8.0, and −13.9 kcal/mol, respectively.

3.4. Analysis of ADMET Profiles. On predictive pkCKSM, the value of logKp greater than −2.5 indicates low skin permeability. If compounds have a logPapp value greater than 0.90, then they are considered to have high Caco-2 permeability. Another important parameter, the volume of distributions (VD), is low if logVD is less than −0.15 and high if it is more significant than 0.45 [48]. It has an impact on total clearance and half-life. Additionally, compounds with log PS > −2 would penetrate the central nervous system (CNS) and act as CNS-active drugs. However, these protease and polymerase inhibitors were found to be central nervous system inactive, so they could be good drugs against DENV infection. Different

TABLE 1: Physicochemical properties of dengue protein.

Physicochemical properties	NS5 (PDB ID: 2J7U)	NS2B/NS3 (PDB ID: 2FOM)	
		Chain A	Chain B
Molecular weight	73489.78	6405.85	19909.62
Theoretical pI	8.01	3.99	8.07
Molecular formula	$C_{3256}H_{5202}N_{892}O_{989}S_{38}$	$C_{265}H_{425}N_{75}O_{105}S_2$	$C_{896}H_{1409}N_{241}O_{268}S_2$
Total no. of atoms	10377	872	2816
Instability index	34.50	83.86	32.42
Aliphatic index	70.17	74.03	79.51
GRAVY	−0.640	−0.555	−0.409

TABLE 2: The prediction of the secondary structure of dengue protein.

Protein name	Alpha helix (Hh)	Extended strand (Ee) (%)	Beta turn (Tt) (%)	Random coil (Cc) (%)
NS5	45.04	13.39	5.20	36.38
NS2B/NS3 (chain A)	38.71	19.35	8.06	33.87
NS2B/NS3 (chain B)	11.35	31.89	15.14	41.62

TABLE 3: H-bonding interaction of nelfinavir and natural metabolites with DENV NS2B/NS3 protease (2FOM) with bond length, interacting residues, GOLD fitness score, and experimental IC_{50} values.

S.N.	Compound name	GOLD fitness score	Calculated binding energy (ΔG) (kcal/mol)	IC_{50} value (μM)	H-bonding residues within $4A^O$ radius	Bond length	Other interaction with resides
Reference	Nelfinavir	41.21	−8.0	—	Trp 118	1.4	—
1.	Agathisflavone (9)	64.12	−8.6	15–17.5	Thr 149	3.4	—
					Asn 196	3.4	
					Val 175	2.1	
2.	Pectolinarin (30)	59.32	−7.8	—	Asn 196	1.8	—
					Lys 103	1.7	
					Gly 116	2.1	
					Leu 114	3.6/1.3	
					Leu 178	2.1	
3.	Panduratin A (5)	54.07	−7.9	90.4 ± 2.3% at 400 ppm	Ile 194	2.2	—
4.	Peridinin (8)	53.17	−7.9	4–7	Trp 112	1.8	—
					Leu 114	1.6	
5.	Epigallocatechin gallate (1)	49.27	−7.7	11–18	Gly 116	2.8	—
					Leu 178	1.7	
					Ala 193	2.2	
					Asn 181	2.2	
					Trp 118	1.7	
6.	Acacetin-7-O-rutinoside (31)	48.05	−14.2	—	Gly 116	2.6	—
					Asn 181	1.4	
					Ala 193	1.6	
7.	4-Hydroxypanduratin A (6)	45.87	−7	90.4 ± 0.3% at 120 ppm	Lys 103	2.2	—
					Asn 181	1.9	

parameters of ADMET properties of selected potent antidengue compounds are shown in Supplementary Tables 10S–13S. The top-scored compounds were within the categorical range of having good pharmacokinetic properties.

4. Discussion

Plants-derived secondary metabolites have been screened to tackle the problems of various infectious diseases because of their effectiveness, easy accessibility, and believed to have low side effects [49]. Natural products have a wide diversity of secondary metabolites, so they are a good source of pharmacological drug candidates against DENV infections. In *in vitro* and *in vivo* assays, various compounds (Figure 1) from natural sources are shown to have antidengue properties (Table 1S). No chemical compounds have been clinically approved as DENV medicine or drugs to date.

4.1. Antidengue Properties of Some Secondary Metabolites.
An antiviral assay based on cytopathic effects (CPE) of six medicinal plants (*Andrographis paniculata, Citrus limon,*

TABLE 4: H-bonding interaction of balapiravir and natural metabolites with DENV NS5 polymerase (2J7U) with bond length, interacting residues, GOLD fitness score, and experimental IC_{50} values.

S.N.	Compound name	GOLD fitness score	Calculated binding energy (ΔG) (kcal/mol)	IC_{50} value (μM)	H-bonding residues within $4A^O$ radius	Bond length	Other interaction with resides
Reference	Balapiravir	49.42	−12.1	—	Arg 482 Asn 127	1.6/2.3 2.0	—
1.	Pectolinarin (30)	50.60	−8.0	138.78 ± 6.10	Glu 197 Ile 487 Arg 482 Lys 122 Lys 123	2.0/3.3 3.8 1.7/2.6/ 2.6 /2.4	 Π-Cation Π-Cation
2.	Acacetin-7-O-rutinoside (31)	46.10	−13.9	—	Lys 123 Thr 126 Asn 127 Arg 482	3.5 2.5 2.9/2.1 2.2/2.2/ 2.2	 Π-Cation
3.	Caffeoylcalleryanin (15)	43.39	−7.7	6.06 ± 0.99	Asn 127 Ser 481 Arg 482	1.8 3.0 1.9/1.8	—
4.	Naringin (29)	43.30	−9.0	289.75	Trp 485 Ser 486 His 491 Ala 489 Arg 482 Lys 123	2.6 1.5 2.2/3.7 2.1 2.1/2.4/ 2.1 2.4/32	 Π-Cation
5.	Glabranine (13)	40.53	−7.8	70% at 25 μM	Asn 127 Arg 482 Thr 126 Lys 122 Lys 123	2.0 2.3 2.1 – –	 Π-Cation Π-Cation
6.	7-O-Methylglabranine (14)	39.86	−7.5	70% at 25 μM	Arg 482	1.7/2.5	—
7.	Anacolosine (18)	39.84		2.5 ± 0.1	Glu 183	1.7	—

Cymbopogon citratus, Momordica charantia, Ocimum sanctum, and *Pelargonium citrosum*) on DENV-1-infected Vero E6 cells with MNTD showed that *A. paniculata,* followed by *M. charantia,* shows significant antiviral inhibitory effects in *in vitro* assays [50]. Extracts of *Boesenbergia rotunda, Carica papaya, Cissampelos pareira, Cladogynos orientalis, Dryopteris crassirhizoma, Eubhorbia hirta, Faramea hyacinthine, Faramea truncate, Flagellaria indica, Gastrodia elata, Houttuynia cordata, Lithospermum erythrorhizon, Morus alba, Myristica faltu, Ocimum sanctum, Piper retrofractum, Psidium guajava, Quercus lusitanica, Rhizophora apiculata, Solanum nigra, Smilar glabla, Syzgium grande, Syzgium companula, Taraxacum officinale, Urtica dioica,* and *Zostera marina* have shown protective activity against different serotypes of DENV in *in vitro* assays [51–58].

4.2. Protease Inhibitors. Molecules capable of inhibiting or altering the virus's protease could be potential drug candidates. Epigallocatechin gallate (1) from *Camellia sinensis* (L.) Kuntze shows antiviral activity with an EC_{50} of 14.8 ± 2.6, 18.0 ± 1.0, 11.2 ± 1.7, and 13.6 ± 0.0 μM for DENV-1, DENV-2, DENV-3, and DENV-4, respectively, in Vero cells, probably by disturbing protein configuration involved in viral infection [59]. An alkaloid, 5-hydroxy-N-methylseverifoline (2) isolated from *Atalantia buxifolia* (Poir.) Oliv. ex Benth. Twigs, demonstrated antiviral activity with an IC_{50} of 5.3 ± 0.4 μM on DENV-infected Huh-7 cells [60]. Pinostrobin (3), cardamonin (4), panduratin A (5), and 4-hydroxypanduratin A (6) isolated from *Boesenbergia rotunda* (L.) showed significant inhibition of DENV-2 NS3 protease in an *in vitro* assay [61]. Panduratin A (4) and 4-hydroxypanduratin A (5) showed competitive inhibition, while pinostrobin (3) and cardamonin (4) act as noncompetitive inhibitors toward DENV-2 NS3 protease with smaller K_i values [61]. Castanospermine (7), an alkaloid from *Castanospermum australe* A. Cunn. ex Mudie, inhibits four serotypes of DENV-infected Huh-7 and BHK-21 cells by altering the protein structure responsible for viral secretion [12]. A carotene like compound, peridinin (8), showed protective effects with an IC_{50} of 7.62 ± 0.17, 4.50 ± 0.46, 5.84 ± 0.19, and 6.51 ± 0.30 μM against DENV-1, DENV-2, DENV-3, and DENV-4, respectively [62]. Agathisflavone (9) isolated from *Cenostigma macrophyllum,*
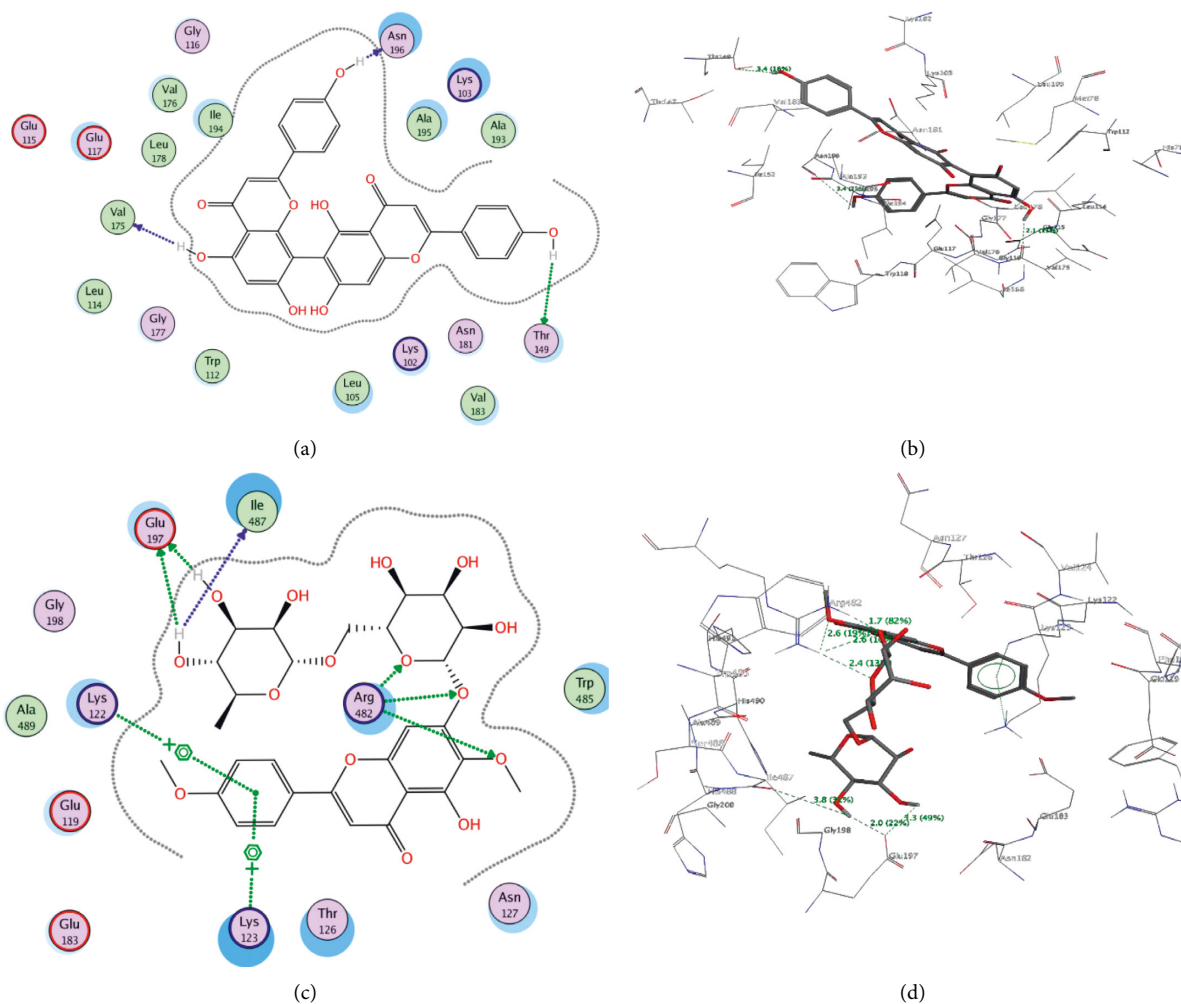
(a)

(b)

(c)

(d)

Figure 2: 2D (a) and 3D (b) interaction of agathisflavone with 2FOM (Fitness score 64.12); 2D (c) and 3D (d) interaction of pectolinarin with 2J7U (Fitness score 50.60). 2FOM is dengue virus NS2B/NS3 protease and 2J7U is dengue virus NS5 RdRP domain.

myricetin (10), and kaempferol (11) showed antiviral activity with an IC_{50} of 15.1 ± 2.2 and $17.5 \pm 1.4\,\mu M$, 22.3 ± 1.8 and $29.3 \pm 3.3\,\mu M$, and 37.8 ± 1.4 and $27.7 \pm 3.2\,\mu M$ against DENV-2 and DENV-3 serine protease, respectively [63].

Phylogenetic analysis showed sequences of NS2B-NS3 and NS5 were highly conserved compared to others [64]. Thus, we have selected them as targets for molecular docking studies of potent antidengue compounds derived from natural products. The present *in silico* study aims to gather information on the probable mechanism of antidengue compounds' action through analysis of ligand-receptor binding.

DENV NS3 is a multifunctional DENV protein (618 amino acid residues) with protease domain at N-terminal, and RNA 5′-triphosphatase, RNA helicase, and nucleoside triphosphates domain in the C-terminal of the peptide, with cofactor NS2B, forms NS3-NS2B complex. It plays a pivotal role in viral polyprotein processing and replication [65–67]. Besides, NS3 also helps in the capping of nascent genomic RNA with an ATPase activity [68]. A catalytic triad (His51-Asp75-Ser135) present between two β-barrels of NS3 is highly conserved and is of prime functional importance [31]. The protein NS2B is involved in several internal cleavages

and cleaving of NS2A/NS2B and NS2B/NS3 [69]. Agathisflavone (9), Pectolinarin (30), Panduratin A (5), Peridinin (8), Epigallocatechin gallate (1), and Pinostrobin (3) fit on the active site of protease (Arg482, Asn127, Glu197, Ile 487, Trp 485, Ser 486, His491, Ala489, Lys123, Thr126, Ser 481) competitively and block the enzyme activity acting, as potential inhibitors of NS3-NS2B protease of DENV. Common interacted active residues were Thr149, Asn196, Val175, Lys103, Gly116, Leu114, Leu178, Ile194, Trp112, Leu114, Gly 116, Ala193, Asn181, and Trp118. Interaction via hydrogen bonds has a noteworthy impact on drug specificity, metabolism, and adsorption [70].

4.3. Replication Inhibitors. Coumarin (12), Glabranine (13), and 7-O-methylglabranine (14) act as replication inhibitors, showing inhibition of 70% DENV-2 infection at $25\,\mu mol/L$ on infected LLC-MK2 cells [71–73]. Caffeoylcalleryanin (15), verbascoside (16), and ursolic acid (17) isolated from the ethanol extract from *Arrabidaea pulchra* (Cham.) Sandwith leaves were protective against DENV-2 with EC_{50} (2.8 ± 0.4, 3.4 ± 0.4, and $3.2 \pm 0.6\,\mu g/mL$) and SI values (20.0, 3.8, and 3.1), respectively [74]. Anacolosine (18), lupenone (19),

β-amyrone (20), and (S)-sambunigrin (21) were isolated from *Anacolosa pervilleana* Baill showed protective activity against DENV [75]. 5,3′-dihydroxy-6,7,4′,5′-tetramethoxy-flavone (DHTMF) (22) and 5-hydroxy-6,7,3′,4′,5′-pentamethoxyflavone (HPMF) (23) were shown to have antidengue properties with EC_{50} of 5.62 μg/mL and 4.47 μg/mL by using dengue virus-green fluorescent protein (DENV/GFP) replicon, a cell-based model [76]. Baicalin (24) isolated from *Scutellaria baicalensis* Georgi showed intracellular antiviral activity on Vero cells against DENV-2 [77]. A flavonoid, fisetin (25) inhibited DENV-2 replication with $IC_{50} = 43.12$ μg/mL (SI = 5.72) and decreased the RNA levels by 65% at 50 μg/mL [78]. Tatanan A (26), isolated from the ethanol extract of the *Acorus calamus,* showed antiviral activity by inhibiting the early steps of RNA replication of DENV-2 [79]. Molecular docking of artesunic acid and homoegenol derived from *M. fatua*, and andrographolide isolated from *A. paniculata* showed that the compounds are best to inhibit NS5 protein of DENV-2 [56, 80]. Similarly, the compounds suramin, naringin, quercetin, catechin, and hesperidin derived from *P. guajava* exhibited higher affinity against NS5 protein [81].

NS5 (104 KDa) is a peptide with 900 residues containing the N-terminal methyltransferase domain and an RNA-dependent RNA polymerase (RdRP) domain at the C-terminal end, responsible for pathogenesis [82, 83], and about 67% amino acid sequence identity across the four serotypes. Due to the presence of RdRP activity, it has a role in RNA replication, while the N-terminal domain is mainly associated with the capping of viral RNA. The RdRP activity was absent in the host cell while it was present in the viral cell, which indicates that a design of specific inhibitors could be a promising antiviral target with low toxicity [84]. Besides its role in RNA replication and methyltransferase activity, NS5 also has a role in the down-regulation of a host immune interferon response [13, 85, 86]. Pectolinarin (30) and Linarin (31) block the active site (Asn 196, Lys 103, Gly 116, Leu 114, Leu 178, Trp 112, Trp 118, Thr 149, Val 175, Ile 194, Asn 181, Ala 193, and Asn 181) of NS5 RNA-dependent RNA polymerase (RdRP) and alter its configuration such that a substrate-enzyme complex cannot be formed. Hence, enzyme activity is blocked, and replication of the virus is retarded preventing infection.

Besides, some secondary metabolites act as both replication and protease inhibitors against DENV. Coumarin (12) showed antiviral activity against the dengue virus by inhibiting viral proteins required for the virus cycle [73]. Flavonoids like quercetin (27), daidzein (28), and naringin (29) isolated from medicinal plants showed antidengue activity with an IC_{50} value of 35.7, 142.6, and 168.2 μg/mL, respectively, on DENV-2 infected Vero cells [87]. Quercetin (27) decreased DENV-2 RNA levels by 67% by inhibiting RNA polymerase in the viral foci reduction assay [87]. Pectolinarin (30) and acacetin-7-O-rutinoside (31) isolated from *Distictella elongata* (Vahl) Urb showed protective activity against DENV [88].

Similarly, *Pavetta tomentosa* Roxb. ex Sm., *Tarenna asiatica* (Linn.) Alston., and *Zanthoxylum limonella* (Dennst.) Alston. extracts showed larvicidal, pupicidal, and adulticidal activity against *Aedes aegypti* [89, 90]. Different herbal products (andrographolide, δ-cadinene, calarene, δ-4-carene, eugenol, pellitorine, sesamin, asarinin, and (+)-xanthoxylol-γ,γ-dimethylallylether) were shown effective against the larva of the dengue vector responsible for transmission to humans, another way to get rid of DENV risks and complications [91–94].

4.4. Pharmacokinetic Parameters and Future Direction. Evaluation of pharmacokinetic properties such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters is indispensable in drug discovery. Toxicity prediction of secondary metabolites, computational methods help in gathering information that assists in clinical trials (*in vitro* and *in vivo* assays) of screening and classifying toxicity [95, 96]. The pharmacokinetic properties of natural product compounds need to be determined before recommending them as potential drug candidates. ADMET analysis revealed that high GOLD fitness scored compounds have the optimum value of parameters under study according to Lipinski's rule of five [97, 98]. Further research (*in vitro*, *ex vivo*, and *in vivo* validation experiments) is required to analyze these metabolites' therapeutic efficacy against different serotypes of DENV for the development of new potential drug candidates.

5. Conclusion

From the dawn of human civilization, we have been battling against different fatal diseases, and natural products have stood out as the best approach to medical treatment against various diseases. Even today, natural products are one of the best medicinal alternatives against viral infections. Many antidengue compounds' availabilities in herbal products are subject to further study and research. Some of these antiviral compounds could be our ultimate weapon against DENV infection. With the aid of a computational approach, we hereby perform a further study on the therapeutic efficiency of agathisflavone, pectolinarin, epigallocatechin gallate, pinostrobin, panduratin A, pectolinarin, and acacetin-7-O-rutinoside as potential DENV inhibitors.

List of Abbreviations

AI: Artificial intelligence
DENV: Dengue virus
DENV-1:   Dengue virus serotype 1
DENV-2:   Dengue virus serotype 2
DENV-3:   Dengue virus serotype 3
DENV-4:   Dengue virus serotype 4
DENV-5:   Dengue virus serotype 5
EC_{50}:   Half maximal effective concentration
GOLD: Genetic optimization for ligand docking
IC_{50}:   Half maximal inhibitory concentration
LD_{50}:   Median lethal dose
MOE: Molecular operating environment
PDB: Protein Data Bank
SI: Selectivity index
WHO: World Health Organization.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Bibek Raj Bhattarai and Bikash Adhikari contributed significantly to the literature review; Bibek Raj Bhattarai, Bikash Adhikari, Asmita Shrestha, Rishab Marahatha, Babita Aryal, and Saroj Basnet performed the in silico analysis; Binod Rayamajhee, Pramod Poudel, and Niranjan Parajuli contributed to editing and revising the manuscript. Bibek Raj Bhattarai and Bikash Adhikari have written and contributed equally to the manuscript. Niranjan Parajuli supervised the project.

Supplementary Materials

Table 1S: some antidengue compounds from natural products; Table 2S: details about molecular docking platform; Table 3S: list of targets showing the PDB ID, resolution and description of the proteins selected for docking with complexed inhibitor; Table 4S: active Site residues of NS2B/NS3 Protease and NS5 polymerase; Table 5S: H-bonding interaction of nelfinavir and natural metabolites with DENV NS2B/NS3 Protease (2FOM) with bond length, interacting residues, GOLD fitness score, and experimental IC_{50} values; Table 6S: H-bonding interaction of balapiravir and Natural metabolites with DENV NS5 Polymerase (2J7U) with bond length, interacting residues, GOLD fitness score, and experimental IC_{50} values; Table 7S: molecular Properties of standard compounds and selected secondary metabolites; Table 8S: binding free energy calculations for NS2B/NS3 Protease with inhibitors using semi-empirical method; Table 9S: binding free energy calculations for NS5 polymerase with inhibitors using semi-empirical method; Table 10S: Prediction of toxicity of selected compounds from natural products by ProTox-II; Table 11S: ADMET properties of NS3 protease inhibitors by pkCSM server; Table 12S: ADMET properties of NS5 polymerase inhibitors by pkCSM server; Table 13S: ADMET properties of NS3 protease and NS5 polymerase inhibitors by pkCSM server; Figure 1S: (A), (B), and (C) represents the graph of secondary structure prediction of proteins NS5, NS2B/NS3 (chain A), and NS2B/NS3 (chain B) respectively analyzed using SOPMA server where blue, red, green and purple colored line represents alpha helix, extended strand, beta turn, and random coil respectively: Figure 2S: 2D upper and 3D lower interaction of nelfinavir with 2FOM (Fitness score 41.21); Figure 3S: upper 2D and lower 3D interaction of pectolinarin with 2FOM (Fitness score 59.32); Figure 4S: Upper 2D and lower 3D interaction of panduratin A with FOM (Fitness score 54.07); Figure 5S: upper 2D and lower 3D interaction of peridinin with 2FOM (Fitness score 53.17); Figure 6S: 2D upper and 3D lower interaction of balapiravir with 2J7U (fitness score 49.42); Figure 7S: upper 2D and lower 3D interaction of naringin with 2J7U (fitness score 43.30). (*Supplementary Materials*).

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