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A N I N T E R N A T I O N A L J O U R N A L

Probing the Pharmacological Parameters, Molecular Docking and Quantum Computations of Plant Derived Compounds Exhibiting Strong Inhibitory Potential Against NS5 from Zika Virus

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ABSTRACT

Zika virus (ZIKV) is known for microcephaly and neurological disease in humans and the nonstructural proteins of ZIKV play a fundamental role in the viral replication. Among the seven nonstructural proteins, NS5 is the most conserved and largest protein. Two major functional domains of NS5 i.e. methyltransferase (MTase) and RNA-dependent RNA polymerase (RdRp) are imperative for the virus life cycle and survival. The present study explicates the inhibitory action of phytochemicals from medicinal plants against NS5 from ZIKV, leading to the identification of potential inhibitors. The crystal structure of the protein is retrieved from RCSB protein data bank. A total of 2035 phytochemicals from 505 various medicinal plants are analysed for their pharmacological properties and pharmacokinetics. Compounds having effective drug-likeness are docked against the protein and further analysed using density functional theory approach. Among the 2035 phytochemicals, 13 are selected as potential inhibitors against MTase having high binding affinities and 17 compounds are selected for RdRp. HOMO and LUMO energies are calculated for the docked compounds within and outside binding pockets of MTase and RdRp, adapting the B3LYP hybrid exchange-correlation functional with def2-SV(P) basis set. Physicochemical properties such as ionization energy, electronic chemical potential, electronegativity, electron affinity, molecular softness, molecular hardness and electrophilicity index have also been analysed for selected phytochemicals. Based upon the results, it is concluded that the selected phytochemicals are highly competent to impede the replication of the virus by inhibiting the ZIKV-NS5.

Keywords: ZIKV-NS5; Phytochemicals; Molecular Docking; DFT calculations;

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INTRODUCTION

Zika infection has emerged as a severe threat to human as it leads to critical, although rare, issues including microcephaly and neurological disease[1]. The source of this infection is Zika virus (ZIKV) which is an arbovirus and belongs to the genus *Flavivirus* of the family *Flaviviridae*[2]. Transmission of ZIKV is carried out through *Aedes spp.* mosquitoes including *Ae. Apicoargenteus*, *Ae. africanus*, *Ae. aegypti*, *Ae. furcifer*, *Ae. hensilli*, *Ae. luteocephalus*, and *Ae. vitattus.* The sexual transmission of disease is also possible as the replicative ZIKV is observed to be found in the semen of an infected man[3]. Furthermore, it is known to be transmitted through perinatal and trans-placental ways as the RNA of ZIKV is detected in the amniotic fluids samples from the fetuses of infected mothers. The infections associated with ZIKV are typically characterized by maculopapular rash, headache, fever, myalgia and conjunctivitis[4].

The genome of ZIKV is a single stranded positive sense RNA which translates into a single polypeptide[5]. The polypeptide is truncated into ten proteins classified as structural and nonstructural proteins. Among those ten proteins, there are three structural proteins i.e. Envelope (E), Membrane (M) and Capsid (C) and seven nonstructural proteins i.e. NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5[6].

NS5 is the largest protein in the genome of ZIKV, consisting of two major domains i.e. a methyltransferase (MTase) domain at residues 1-262 and an RNA dependent RNA polymerase (RdRp) at residues 273-903. There is also an inter-domain region at residues 263-272[7]. MTase domain of the protein is a key target for drug discovery as it performs an essential role in the replication of the virus i.e. nucleoside-2'O and N-7 methylation of the viral RNA cap. Unfavourable effects can be observed on the progression of ZIKV by inhibition of MTase[8]. RdRp domain of ZIKV-NS5 initiates the RNA synthesis and generates positive and negative strand RNAs. The structure of RdRp resembles a shape analogous to hand, comprising finger thumb and palm region. There is no RdRp enzyme or its analogue structure in the human body, therefore the inhibitors of this enzyme do not cause any toxicity in the body by interacting with any other enzyme which makes this domain an optimal target of for drug designing[9-10].

To date, there exists no clinical antiviral treatment against ZIKV. New research methods are adapted for identification of inhibitors against this virus[3]. ZIKV is known to target the neuronal cell and it is necessary for a drug to have blood brain barrier (BBB) permeability[11]. Through analysing the pharmacological properties and pharmacokinetics of compounds, i.e. Absorption, Distribution, Metabolism. Excretion and Toxicity, the safe and effective disposition of compound in the human body can be assessed. Those assessed compounds can be docked against target proteins easily using computational docking mechanism. It makes the process of finding inhibitors efficient as it does not requires laborious work and cost in terms of resources or money. Analysis of chemical reactivity descriptors using DFT is very important to understand chemical and biological activities of the compounds against the biological target. It helps in characterizing the electrophilic or nucleophilic nature, their ionization, potential and affinity towards the targets. Phytochemicals are produced in biosynthetic pathways of the plants, and there is a huge variety of these compounds which are known to have potential to be used against many diseases in humans[12]. The metabolic products of the plants have been reported to be having antiviral, antibacterial, antifungal, anticancer, and many other activities[13]. The present study elucidates the potential of phytochemicals from various medicinal plants, inhabited in Pakistan and India. These compounds are docked against NS5 from Zika Virus (ZIKV) to cease the replication of the virus.

MATERIAL AND METHODS

The structures of NS5 MTase and RdRp domains were retrieved from Protein Data Bank (PDB ID: 5M5B and 5U04, respectively). A total of 2035 plant-derived phytochemicals were analysed in this study (Table 1). The compounds belonged to a variety of medicinal plants of Pakistan and India. These compounds were searched from literature review and structures were retrieved from PubChem. Initial screening was based on pharmacologic and pharmacokinetic properties to select BBB-permeable compounds. Screened compounds are docked and interactions are analysed for potentially inhibiting compounds. Molecular orbital energies based physiochemical properties are also analysed for these compounds, within and outside the pocket of proteins.

Table 1: Details of phytochemicals set

Phytochemical group	Compounds
Alkaloids	322
Terpenoids	113
Aurones	105
Chalcones	101
Flavonoids	378
Lignans	211
Carboxylic acids	255
Polyphenols	301
Quinones	249

Assessing pharmacological properties of phytochemicals

Pharmacological properties i.e. Absorption, Distribution, Metabolism and Excretion along with the prediction of Toxicity were used for screening the phytochemicals. These parameters also helped in evaluating the drug likeness effective disposition of the phytochemicals in the human body. The prediction of ADME profiles was carried out using SwissADME web server[14]. Toxicity and the Drug likeness was predicted using the PreADMET server[15].

Molecular docking and validation of results

The docking study was performed to analyse the inhibition role of phytochemicals against both domains of NS5 protein i.e. MTase and RdRp. The ligand and protein preparation was performed in AutoDock Tools and docking was performed using AutoDock Vina[16,17]. The interactions of the compounds were analysed along with the estimation of binding energies and for further analysis of docking, K_i was calculated by the equation:

$$K_i = e^{\frac{\Delta G}{R\,x\,T}}$$

Where ΔG is docking energy, R (gas constant) is 1.9872036 cal/mol and T (temperature) is 298.15K. For validation of results and selection of potential inhibitors, natural ligands of both domains i.e. Guanosine Triphosphate (GTP) and S-Adenosyl methionine (SAM) were docked with RdRp and MTase, respectively; and binding energies were compared. Compounds showing strong binding affinity as compared to the known ligands were further analysed for DFT analysis.

DFT study of phytochemicals and band energy gap analysis

Lowest Unoccupied Molecular Orbital (LUMO) and Highest Occupied Molecular Orbital (HOMO) energies of the phytochemicals were calculated using ORCA program by applying the Becke3-Lee-Yang-Parr (B3LYP) hybrid functional exchange correlation of DFT to estimate the ionization energy, electronic chemical potential, electronegativity, electron affinity, molecular softness, molecular hardness and electrophilicity index[18]. For these calculations, following equations were used.

$$Ionization\ energy\ (IE) = -\epsilon_{HOMO}$$
$$Electron\ affinity\ (EA) = -\epsilon_{LUMO}$$
$$Electronegativity\ (\chi) = \frac{(IE + EA)}{2}$$
$$Electronic\ chemical\ potential\ (\mu) = -\chi$$
$$Chemical\ hardness\ (\eta) = \frac{(IE - EA)}{2}$$
$$Chemical\ softness\ (\sigma) = \frac{1}{\eta}$$
$$Electrophilicity\ index\ (\omega) = \frac{\mu}{2\eta}$$

The targeted hybrid exchange correlation i.e. B3LYP is defined as:

$$E_{xc}^{B3LYP} = E_x^{LDA} + a_0\,(E_x^{HF} - E_x^{LDA}) + a_x\,(E_x^{GGA} - E_x^{LDA}) + E_c^{LDA} + a_c\,(E_c^{GGA} - E_c^{LDA})$$

Where $a_0 = 0.20$, $a_x = 0.72$, and $a_c = 0.81$. E_x^{GGA} is actually the generalized gradient approximation for the Becke 88 functional while the E_c^{GGA} reflects the correlation functional of Lee-Yang-Parr. E_c^{LDA} is the local density approximation. To analyse the reactivity of phytochemicals in the binding pocket of MTase and RdRp, band energy gaps were calculated using the expression E_{LUMO}-E_{HOMO}[19,20]. Phytochemicals along with the interacting residues from binding pocket were considered for the calculations and for each phytochemical, the conformer with maximum binding energy was analysed.

RESULTS AND DISCUSSION

ZIKV infection is an evolving threat to humans and has long term detrimental effects in terms of microcephaly, male sterility and neurological disease. The inhibition of ZIKV is one of the major concerns of the clinical and scientific community. Tertiary structure of ZIKV-NS5 reveals an N-terminal MTase domain, situated at the top of C-terminal RdRp domain. A Rossmann fold dominates the MTase domain with a seven stranded β-sheet, between two α-helices[21]. To find the potential inhibitors, a total of 2035 phytochemicals were analysed for in this study for their inhibitory potential against ZIKV-NS5.

Pharmacological properties of phytochemicals

All 2035 compounds were analysed for their pharmacological properties and pharmacokinetics. Properties being analysed included solubility, gastrointestinal (GI) absorption, blood brain barrier (BBB) permeability, Lipinski's rules violation and toxicity. The criteria set for selecting suitable compounds was:

(BBB-permeability = Yes) & (Solubility = High) & (GI-absorption = High) & (Lipinski's violations = 0) & (Toxicity = No)

At each level of criteria set, the number of compounds selected for further analysis was reduced. Initially, out of 2035 phytochemicals, only 720 were BBB permeable. Due to the association of ZIKV with neuronal infection, it was necessary to select only those compounds which can penetrate through BBB[22]. From those 720 compounds, only 344 had high solubility and GI absorption. Among 344 compounds, 280 violated no rule of Lipinski and at the end, only 153 were left as non-toxic phytochemicals (Table S1). All of these parameters are necessary to evaluate the drug-likeness of a compound and helps in assessing the effective disposition of compounds in the human body[23-26].

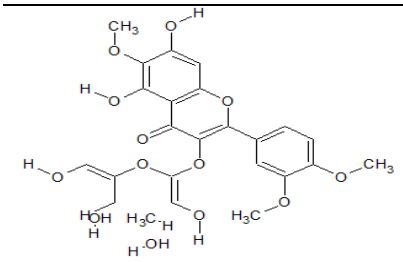
Analysis of dockings and interactions

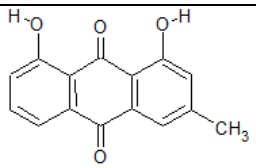
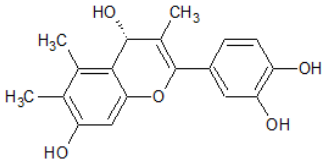
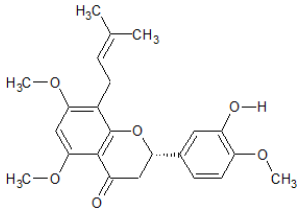
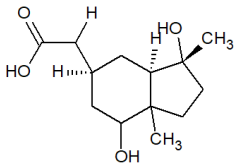
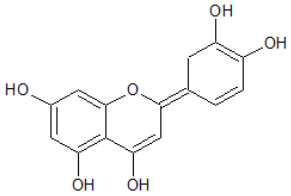
153 compounds, screened on the basis of pharmacological properties, were docked against both the domains of ZIKV NS5 i.e. MTase and RdRp. SAM and GTP were docked against MTase and RdRp, respectively, as they both are reported as natural ligands of the MTase and GTP[27]. SAM docked with MTase having a binding affinity of -7.7 kcal/mol (K_i = 2.24 µM) whereas binding affinity of GTP was -6.9 kcal/mol (K_i = 8.64 µM) against RdRp. From 153 phytochemicals, 13 phytochemicals showed binding affinity greater than or equal to -7.7 kcal/mol against MTase and 17 phytochemicals showed binding affinity greater than -6.9 kcal/mol against RdRp.

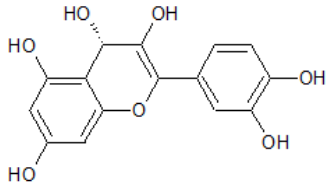
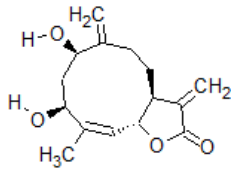
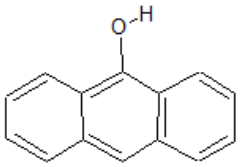
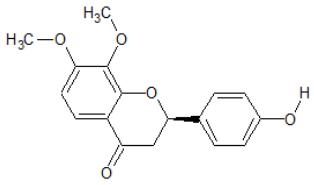
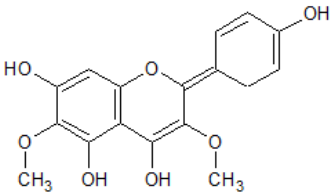
Docking phytochemicals against MTase

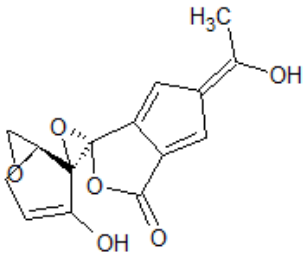
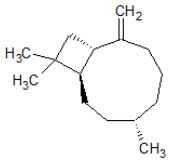
153 compounds were docked against MTase and 13 showed binding affinity greater than the binding affinity of SAM, a natural ligand for MTase domain of NS5. Results showed that Quercetagetin, a flavonol, docked with viral enzyme having a binding affinity -10.9 kcal/mol (K_i = 0.01 µM) which is greater than affinities of all other compounds (Table 2). The compound docked in the binding pocket of MTase interacted with Val_{30}, Val_{32}, His_{110} and His_{111} (Fig. 1). Chrysophanic Acid (a quinone) and Jaceidin (a flavonoid) also docked with good binding affinities i.e. -10.2 kcal/mol (K_i = 0.03 µM) and -10.1 kcal/mol (K_i = 0.04 µM), respectively. Chrysophanic acid interacted with Val_{30}, Val_{32} and Ile_{147} at the binding pocket of MTase while Gly_{81}, Asp_{146} and Phe_{133} were involved in the interactions with Jaceidin.

Table 2: Phytochemicals strongly inhibiting ZIKV-MTase

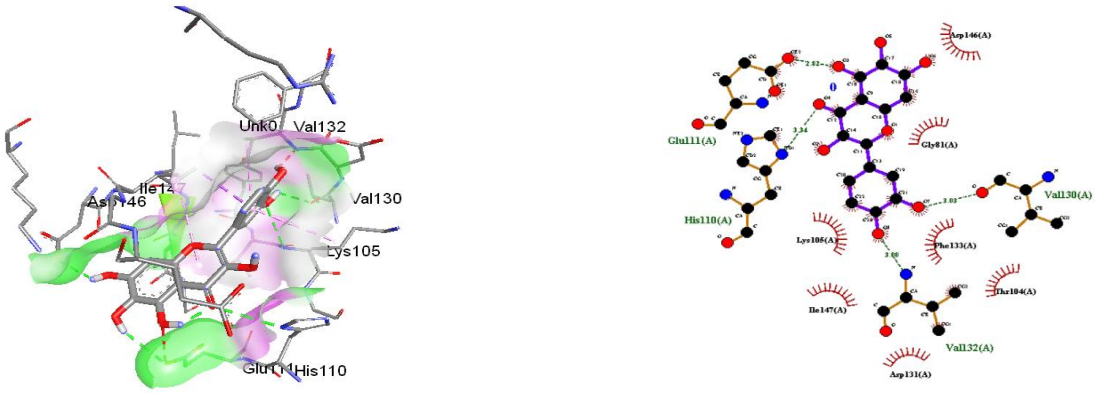
Phytochemicals	**Interacting Residues**	**Binding energy (MTase) (kcal/mol)**	**K_i (µM)**
 Quercetagetin	Val_{30}, Val_{32}, His_{110}, Glu_{111}	-10.9	0.01

Phytochemicals	Interacting Residues	Binding energy (MTase) (kcal/mol)	K_i (µM)
 Chrysophanic Acid	Val_{30}, Val_{32}, Ile_{147}	-10.2	0.03
 Jaceidin	Gly_{81}, Asp_{146}, Phe_{133}	-10.1	0.04
 Methylglovanon	Lys_{105}, Asp_{131}, Ile_{147}	-9.5	0.11
 Caninnot	Thr_{104}, Asp_{131}, Phe_{133}	-8.9	0.29
 Luteolin	Val_{30}, Gly_{81}, Glu_{111}	-8.6	0.49

Phytochemicals	Interacting Residues	Binding energy (MTase) (kcal/mol)	K_i (µM)
 Quercetin	His_{110}, Glu_{111}, Asp_{131}	-8.6	0.49
 Redentin	Val_{30}, Val_{32}, Phe_{133}	-8.6	0.49
 Anthranol	Lys_{105}, Phe_{133}, Ile_{147}	-8.4	0.69
 HeliannoneB	Gly_{81}, Asp_{146}, Ile_{147}	-8.4	0.69
 Hydroxy Kemferol	Lys_{105}, Phe_{133}	-8.2	0.96

Phytochemicals	Interacting Residues	Binding energy (MTase) (kcal/mol)	K_i (µM)
 Secterpathe	His_{110}, Glu_{111}, Thr_{104}, Asp_{131}	-7.9	1.60
 Beta Caryophylene	Val_{32}, Gly_{81}	-7.8	1.89

Previously, ZIKV-MTase has been targeted using various compounds. Byler *et al*., (2016) reported that cimiphenol, cimiracemate B, and rosemarinic acid exhibited relatively strong docking energies as among 2263 phytochemicals docked against MTase in that study[28]. ZINC library compounds were analysed against ZIKV-MTase and results of docking were compared with BG323, a known inhibitor of MTase. It is reported that ZINC64717952 showed high binding affinity against MTase as compared to that of BG323[3].



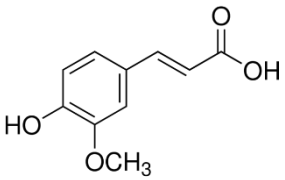
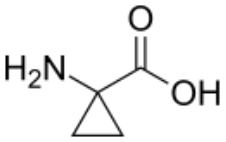
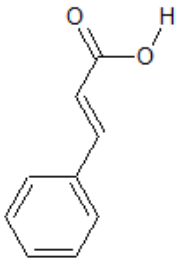
(a) **(b)**

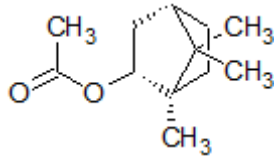
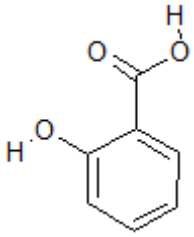
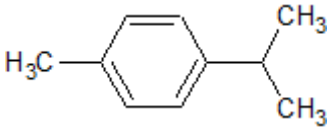
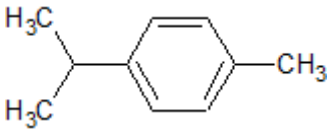
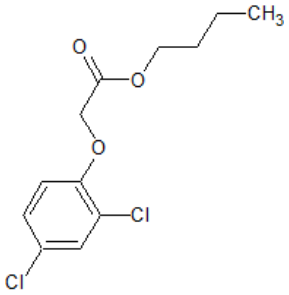
Figure 1: Interaction of Quercetagetin within binding pocket of ZIKV MTase **(a)** 3D view of pocket **(b)** Interaction plot

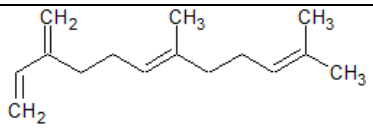
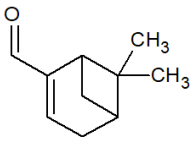
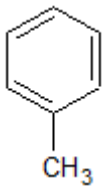
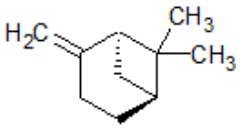
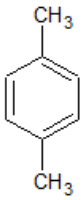
Docking phytochemicals against RdRp

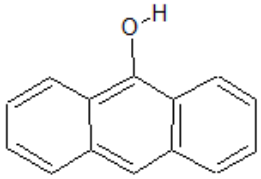
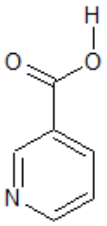
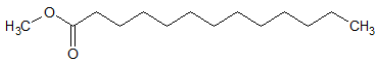
Among all 153 compounds docked against RdRp, 17 showed binding affinity greater than the binding affinity of GTP, a natural ligand for RdRp domain of NS5. Results showed that Ferulic Acid, a phenol, docked with binding affinity -9.0 kcal/mol, greater than affinities of all other compounds (Table 3). The compound docked in the binding pocket of RdRp interacting with Tyr_{609}, Ile_{799} and His_{800} (Fig. 2). 1-Aminocyclopropane-1-carboxylic acid and Cinnamic acid, both carboxylic acids in nature, docked with binding affinities -8.9 kcal/mol (K_i = 0.29 µM) and -8.8 kcal/mol (K_i = 0.35 µM), respectively. 1-Aminocyclopropane-1-carboxylic acid interacted with Tyr_{609}, Asn_{612}, Ser_{798}, Ile_{799} and His_{800} residues at the RNA tunnel of ZIKV-RdRp. Moreover, Cinnamic acid docked at the RNA tunnel of RdRp by interacting with Tyr_{609}, Asn_{612}, Ser_{663}, Asp_{665}, Asp_{666} and Cys_{711}.

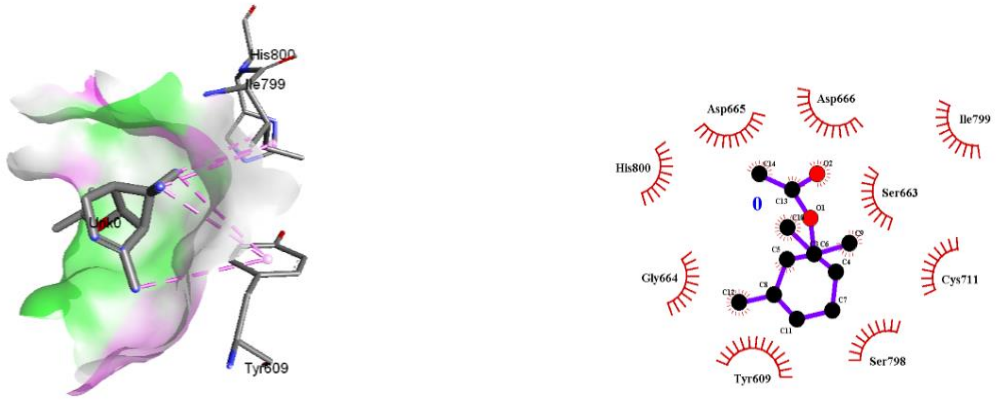
Table 3: Phytochemicals strongly inhibiting ZIKV-RdRp

Phytochemicals	Interacting residues	Binding energy (RdRp) (kcal/mol)	Ki (µM)
 Ferulic Acid	Tyr_{609}, Asn_{612}, Ser_{663}, Gly_{664}, Asp_{665}, Asp_{666}, Cys_{711}, Ser_{798}, Ile_{799}, His_{800}	-9.0	0.25
 1-Aminocyclopropane-1-carboxylic acid	Tyr_{609}, Asn_{612}, Ser_{798}, Ile_{799}, His_{800}	-8.9	0.29
 Cinnamic Acid	Tyr_{609}, Asn_{612}, Ser_{663}, Asp_{665}, Asp_{666}, Cys_{711}	-8.8	0.35

Phytochemicals	Interacting residues	Binding energy (RdRp) (kcal/mol)	Ki (µM)
 Bornylacetate	Gly_{664}, Asp_{665}, Asp_{666}, Cys_{711}, Ser_{798}, His_{800}	-8.6	0.49
 Salicylic Acid	Cys_{711}, Ser_{712}, Trp_{797}, Ile_{799}	-8.5	0.58
 Cymene	Met_{660}, Ala_{661}, $Val_{662,}$ Cys_{711}, Ser_{798}	-8.4	0.69
 Gamma Terpinene	Tyr_{609}, Ile_{799}, His_{800}	-8.4	0.69
 Kolin	Ser_{663}, Gly_{664}, Asp_{665}, Cys_{711}, Ser_{798}	-8.2	0.96

Phytochemicals	Interacting residues	Binding energy (RdRp) (kcal/mol)	Ki (µM)
 Fernesene	Asp_{666}, Cys_{667}, Val_{668}, Ser_{798}, His_{800}	-8.0	1.35
 Myrtenal	Met_{660}, Val_{662}, His_{800}	-8.0	1.35
 Alphaprodine	Asn_{612}, Gly_{664}, Thr_{796}	-7.9	1.60
 Beta Pinene	Asn_{612}, Gly_{664}, Thr_{796}	-7.9	1.60
 Xylene	Val_{662}, Cys_{711}, Ser_{798}	-7.8	1.89

Phytochemicals	Interacting residues	Binding energy (RdRp) (kcal/mol)	Ki (µM)
 Benzaldehyde	Asn_{612}, Asp_{666}, Cys_{711}, Ser_{798}	-7.6	2.65
 Nicotinic Acid	Glu_{509}, Gly_{664}, Thr_{796}	-7.5	3.14
 Tridecanoic Acid	Gly_{507}, Val_{508}	-7.4	3.71
 Cyclopentadiene	Cys_{711}, His_{800}	-7.0	7.30



(a) **(b)**

Figure 2: Interaction of Ferulic Acid within RNA Tunnel of ZIKV RdRp **(a)** 3D view of pocket **(b)** Interaction plot

ZIKV-RdRp is a potential target for drug discovery as RdRp enzyme or its analogue structure in the human body are not present, therefore the inhibitors of this enzyme do not cause any toxicity in the body by interacting with any other enzyme[9]. In a previous study, Prenylatedchalcone, 2',4,4'-trihydroxy-3,3'-diprenylchalcone, bisindole and flinderoleB are reported to potentially inhibit ZIKV-RdRp domain with strong binding affinities. Among 2263 phytochemicals, these four compounds were screened based on high binding energies[28]. While targeting this enzyme with ZINC library, it was observed that ZINC39563464 showed the strong binding affinity to RdRp. The score was also higher as compared to that of Ribavirin, a potential inhibitor of RdRp[3]. In the present study, Quercetagetin was docked with MTase, having highest binding affinity as compared to that of all other compounds, however, in another study, Quercetagetin was reported as inhibitor of HCV-NS5B, which is also an RdRp[29]. This illustrates that Quercetagetin showed binding affinity < -6.9kcal/mol when docked with ZIKV-RdRp.

Quantum mechanistic analysis of selected 30 compounds

30 phytochemicals showed strong inhibitory potential against the NS5 protein of ZIKV and were selected for further analysis. Two types of DFT studies were conducted for these compounds i.e. within the binding pockets and outside the binding pockets of NS5. Ionization energy, electronic chemical potential, electronegativity, electron affinity, molecular softness, molecular hardness and electrophilicity index were calculated by targeting the compounds only (Table 4).

Table 4: Quantum mechanistic analysis of phytochemicals

Phytochemicals	LUMO (eV)	HOMO (eV)	Ionization Energy (eV)	Electron Affinity (eV)	Electronegativity (eV)	Electronic chemical potential (eV)	Hardness (eV)	Softness (eV)	Electrophilicity Index
1-Aminocyclopropane-1-carboxylic acid	-0.245	-0.365	0.365	0.245	0.305	-0.305	0.060	16.667	-2.542
Alphaprodine	-0.194	-0.319	0.319	0.194	0.257	-0.257	0.063	16.000	-2.052
Anthranol	-0.238	-0.369	0.369	0.238	0.304	-0.304	0.066	15.267	-2.317
Benzaldehyde	-0.179	-0.304	0.304	0.179	0.242	-0.242	0.063	16.000	-1.932
Beta Caryophylene	-0.202	-0.334	0.334	0.202	0.268	-0.268	0.066	15.152	-2.030
Beta Pinene	-0.296	-0.421	0.421	0.296	0.359	-0.359	0.063	16.000	-2.868
Bornylacetate	-0.260	-0.380	0.380	0.260	0.320	-0.320	0.060	16.667	-2.667
Caninnot	-0.202	-0.325	0.325	0.202	0.264	-0.264	0.062	16.260	-2.142
Chrysophanic Acid	-0.232	-0.346	0.346	0.232	0.289	-0.289	0.057	17.544	-2.535
Cinnamic Acid	-0.244	-0.363	0.363	0.244	0.304	-0.304	0.060	16.807	-2.550
Cyclopentadiene	-0.146	-0.295	0.295	0.146	0.221	-0.221	0.075	13.423	-1.480
Cymene	-0.154	-0.275	0.275	0.154	0.215	-0.215	0.061	16.529	-1.773
Fernesene	-0.176	-0.299	0.299	0.176	0.238	-0.238	0.062	16.260	-1.931
Ferulic Acid	-0.209	-0.328	0.328	0.209	0.269	-0.269	0.060	16.807	-2.256
Gamma Terpinene	-0.214	-0.336	0.336	0.214	0.275	-0.275	0.061	16.393	-2.254
HeliannoneB	-0.194	-0.326	0.326	0.194	0.260	-0.260	0.066	15.152	-1.970
Hydroxy Kemferol	-0.128	-0.260	0.260	0.128	0.194	-0.194	0.066	15.152	-1.470
Jaceidin	-0.175	-0.290	0.290	0.175	0.233	-0.233	0.058	17.391	-2.022
Kolin	-0.257	-0.379	0.379	0.257	0.318	-0.318	0.061	16.393	-2.607
Luteolin	-0.293	-0.422	0.422	0.293	0.358	-0.358	0.065	15.504	-2.771
Methylglovanon	-0.167	-0.289	0.289	0.167	0.228	-0.228	0.061	16.393	-1.869
Myrtenal	-0.158	-0.282	0.282	0.158	0.220	-0.220	0.062	16.129	-1.774
Nicotinic Acid	-0.253	-0.378	0.378	0.253	0.316	-0.316	0.063	16.000	-2.524
Quercetagetin	-0.230	-0.341	0.341	0.230	0.286	-0.286	0.056	18.018	-2.572
Quercetin	-0.211	-0.341	0.341	0.211	0.276	-0.276	0.065	15.385	-2.123

Redentin	-0.292	-0.423	0.423	0.292	0.358	-0.358	0.066	15.267	-2.729
Salicylic Acid	-0.160	-0.281	0.281	0.160	0.221	-0.221	0.061	16.529	-1.822
Secterpathe	-0.197	-0.329	0.329	0.197	0.263	-0.263	0.066	15.152	-1.992
Tridecanoic Acid	-0.272	-0.421	0.421	0.272	0.347	-0.347	0.075	13.423	-2.326
Xylene	-0.230	-0.355	0.355	0.230	0.293	-0.293	0.063	16.000	-2.340

Among all the compounds, Quercetagetin showed the most effective properties i.e. hardness (0.056 kcal/mol), electrophilicity index (-2.572 kcal/mol) and electronic chemical potential (-0.286 kcal/mol). Chemical hardness of a compound illustrates its stability and reactivity. The larger band energy gaps reflect hardness and stability of the compounds but leads to lower reactivity. Smaller band energy gaps reflect softness and high reactivity of compounds. The electronic chemical potential is actually the negative value of electronegativity and the greater electronic chemical potential reflects less stability and high reactivity of the compounds. The mechanism and capacity of electrons acceptance is measured through electrophilicity index[30,31]. It measures the stabilization in energy after the chemical reaction through electron acceptance and donation[32]. Such analysis has been previously conducted by Gopalakrishnan *et al*., (2014) to analyse the phytochemical constituents of the *Cucumis trigonus* and *Cucumis sativus*[33].

For analysing reactivity, phytochemicals and the binding pocket residues was the point of interest for energy calculations. The quantum mechanistic analysis was carried out on the ligand-NS5 domain conformation selected in the docking analysis. Results revealed that Quercetagetin was most reactive compound within binding pocket of NS5 as band energy gap was narrow as compared to others i.e. 0.119 eV, while the natural ligand i.e. SAM showed least reactivity as band energy gap was 0.173eV, lowest among all the other 13 compounds (Table 5). For RdRp domain, Ferulic Acid had the narrow band energy gap i.e. 0.114 eV reflecting its higher reactivity potential with the binding pocket residues of RdRp, while the natural ligand i.e. GTP showed least reactivity as band energy gap was 0.182eV, lowest among all the other 17 compounds (Table 6).

Table 5: Band energy gaps analysis in the binding pocket of MTase

Phytochemicals	**E_{LUMO} (eV)**	**E_{HOMO} (eV)**	**Band energy gap (ΔE) (eV)**
Quercetagetin	-0.186	-0.305	0.119
Chrysophanic Acid	-0.295	-0.417	0.122
Jaceidin	-0.269	-0.393	0.124
Methylglovanon	-0.220	-0.346	0.126
Caninnot	-0.157	-0.305	0.148
Luteolin	-0.158	-0.306	0.148
Qurecitin	-0.237	-0.387	0.150
Redentin	-0.188	-0.339	0.151
Anthranol	-0.180	-0.332	0.152
HeliannoneB	-0.227	-0.379	0.152
Hydroxy Kemferol	-0.131	-0.290	0.159
Secterpathe	-0.195	-0.360	0.165
Beta Caryophylene	-0.285	-0.455	0.170
S-Adenosyl methionine	-0.291	-0.464	0.173

Table 6: Band energy gaps analysis in the binding pocket of RdRp

Phytochemicals	**E_{LUMO} (eV)**	**E_{HOMO} (eV)**	**Band energy gap (ΔE) (eV)**
Ferulic Acid	-0.274	-0.388	0.114
1-Aminocyclopropane-1-carboxylic acid	-0.153	-0.27	0.117
Cinnamic Acid	-0.137	-0.263	0.126
Bornylacetate	-0.292	-0.426	0.134
Salicylic Acid	-0.305	-0.444	0.139
Cymene	-0.224	-0.364	0.140
Gamma Terpinene	-0.260	-0.401	0.141
Kolin	-0.186	-0.337	0.151
Fernesene	-0.284	-0.436	0.152
Myrtenal	-0.175	-0.327	0.152
Alphaprodine	-0.282	-0.434	0.152
Beta Pinene	-0.221	-0.375	0.154
Xylene	-0.115	-0.272	0.157
Benzaldehyde	-0.119	-0.279	0.160
Nicotinic Acid	-0.256	-0.419	0.163
Tridecanoic Acid	-0.252	-0.417	0.165
Cyclopentadiene	-0.162	-0.333	0.171
Guanosine Triphosphate	-0.169	-0.351	0.182

In literature, no study has been reported previously targeting the analysis of compound reactivity within binding pocket of ZIKV proteins. However, DFT based analysis is reported in some other studies and the band energy gaps of the present study are also within same range of those reported in previous studies[34,35]. The lower band energy gap reflects higher reactivity of compounds as the E_{LUMO} and E_{HOMO} are responsible for the charges transferred in a chemical reaction[36]. In the present study, the thirty screened out phytochemicals were evaluated based upon DFT analysis and the lower band energy gap of molecular orbital energies illustrated the higher reactivity of these phytochemicals[37]. This validates the potential of the thirty phytochemicals against ZIKV-NS5.

CONCLUSION

The infection associated with ZIKV leads to long term effects in terms of various diseases, specifically neurological ones. The Zika infection is an emerging threat and no clinically approved antiviral treatment is discovered yet to cure the disease. Computational approaches for drug development are proven to be effective and time efficient as these are not based on costly and hectic laborious works. In drug discovery, the molecular dynamics simulations help in the study of the motions of biological macromolecules such as proteins and nucleic acids. Computational mechanisms of biological targets and their associated small-molecule ligands can play an important role in drug discovery; including the identification of binding sites, virtual screening of numerous compounds libraries and estimation of ligand binding energies. The docking of inhibitors with the enzymes elucidate the mechanism of inhibition along with the specificity and efficiency of that inhibitor. It was aim of the present study to identify potential inhibitors of the virus replication and the most fundamental protein i.e. NS5 was targeted in this study using 2053 plant derived compounds. The successfully docked compounds with high binding affinity and reactivity are reported against both the domains of NS5 i.e. MTase and RdRp. These are all BBB-permeable and can reach the central nervous system as ZIKV targets the neuronal cells. Due to higher affinities and reactivity of these compounds against NS5, these 30 compounds have potential inhibitory activity against ZIKV and can be further analysed using *in vitro* and *in vivo* approach to elucidate their inhibitory mechanism against ZIKV.

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