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========== **BIOINFORMATICS** ==========

Exploring the Mangrove Based Phytochemicals as Potential Viral RNA Helicase Inhibitors by *in silico* Docking and Molecular Dynamics Simulation Method

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Abstract. The high molecular diversity of plant-derived compounds in mangroves has drawn attention to the discovery of their antiviral capacity against several pathogenic viruses. Therefore, screening for effective antiviral compounds with fewer harmful side effects is needed. This study aimed to screen several bioactive compounds from mangrove plants that could be appropriately used as an RNA helicase inhibitor against pathogenic viruses. Fifty-nine compounds were selected from literature and databases for initial study and screening according to Lipinski's rule of five. The chosen compounds obtained were subjected to another series of screening by molecular docking study with five different RNA helicase enzymes of the pathogenic virus using the Autodock Vina tool, followed by ADMET (absorption, distribution, metabolism, excretion, and toxicity) analysis. In addition, the best compound-bound helicase RNA complexes were included in a 50 nanosecond molecular dynamics simulation using the Gromacs 5.1.1 software, followed by Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) analysis. This comparative study predicts that phytochemical gedunin is an excellent inhibitor of the RNA helicase enzyme of SARS-CoV-2, followed by the Japanese encephalitis virus and hepatitis C virus (HCV). The results of the study may lead to the development of antiviral compounds against RNA helicase enzymes of pathogenic viruses.

Key words: pathogenic viruses, RNA helicase, molecular docking, molecular dynamics simulation, mangrove phytochemicals.

INTRODUCTION

Outbreaks of pathogenic viruses are the source of several infectious diseases and cause loss of life. Most infections are caused by viruses, which develop into pandemics and spread rapidly between people. Examples of severe outbreaks and pandemics are Spanish flu, Hong Kong flu, SARS H7N9, Ebola, Zika, and Covid-19 that have occurred many times [1, 2]. Pathogenic RNA viruses comprise different structural and non-structural proteins (NSPS). Structural proteins like spike, envelope, membrane, and nucleocapsid proteins are involved in viral capsid synthesis. In contrast, NSPS plays an essential role in viral metabolism, such as genome replication. These proteins include polymerase, helicase, and other accessory proteins [3, 4]. Among several non-structural proteins, the enzyme RNA helicase plays a vital role in viral genome replication [5]. The functional importance of this helicase enzyme is that it acts as a motor protein that performs the coiling and untwisting of the RNA genome using adenosine triphosphate (ATP) molecules. Therefore, this enzyme can be used as a potential therapeutic target for developing bioactive molecules to control viral infections [6–8]. However, the experimental basis for designing new molecules against the viral RNA helicase enzyme is difficult. For example, monitoring the real-time cleavage of helicase enzymes and RNA (during metabolism) by high-throughput assay is challenging. Again, the drug molecule should be selected carefully; otherwise, it can compete with the natural substrate (ATP) by binding to the

ATP binding site of the host [9–11]. Therefore, bioinformatics-based techniques can be used to investigate the fact as an alternative method. Several databases and software tools have been reported, and the same can be applied to the screening and evaluation of novel drug molecules against enzymes. Furthermore, because of their low time and cost, in silico methods play an essential role in the computer-aided drug design (CADD) process [12–14]. Computer modeling methods such as molecular docking, molecular dynamics simulations, quantitative structure-activity relationships, and other sophisticated methods are commonly used to find effective drug molecules. Recently, several molecules have been predicted to inhibit the RNA helicase of SARS-CoV2 by computational methods [15, 16]. In addition, a curated database of RNA helicase inhibitors has been developed, containing information on several molecules predicted to be inhibitors of the viral RNA helicase enzyme [17].

Traditionally used antiviral drugs have shown their efficacy in *in vitro* analysis against most viruses but have failed in their activity in patients. Furthermore, these molecules are often expensive and achieve antiviral and harmful side effects. Therefore, natural compounds from plant sources may be a suitable alternative for treating viral infections. It is also necessary to examine their phytochemical type, bioavailability, binding affinity, and ADMET properties, which will highlight their therapeutic applications as antiviral agents. Various phytochemicals have been used in traditional medicine systems since ancient times and are known for their potent therapeutic effects against viral infections [18–20]. Among the different plants, mangroves are the primary source of antiviral compounds. Past studies confirmed that mangrove plants produce a variety of phytochemicals due to their unique ecological adaptability, providing the basis for the search for new therapeutic molecules. These molecules exhibit essential medicinal properties and are widely applied to treat viral, antibacterial, and antifungal diseases [21–23]. The biochemical basis for classifying mangrove phytochemicals includes alkaloids, flavonoids, triterpenoids, polyphenolics, xanthones, coumarins, and tannins [24, 25].

The present work aimed to screen potential phytochemicals from mangrove plant sources and evaluate their efficacy against five viral RNA helicase enzymes using molecular docking, ADMET analysis and molecular dynamics (MD) simulation studies.

MATERIALS AND METHODS

System configuration

Processor: AMD Ryzen 3900*4.6 GHz, Mother Board: Gigabyte B550 Acurs pro AC, RAM: 32 GB, GPU: Asus dual GT 165004 G, Operating system: Ubuntu Version 2021.

Preparation of Ligand and Receptor molecules

Information on specific phytochemicals from mangrove plant sources was searched in various databases such as PubChem (https://pubchem.ncbi.nlm.nih.gov/), IMPPAT (https://cb.imsc.res.in/imppat/) as well as published documentation. Similarly, three-dimensional (3D) structures of five viral helicases were obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/) database (Table 1).

Table 1. Showing the 3D structure of helicase enzymes considered for the study

Name of the virus	Family	PDB IB	No. of Chain	Sequence length	Resolution (A^0)	*ATP binding domain region
Dengue virus	Flaviviridae	2BMF	2	451	2.41	28–118
Zika virus	Flaviviridae	5K8U	1	458	1.60	34–123
Japanese encephalitis virus	Flaviviridae	2Z83	1	459	1.80	30–120
Hepatitis C Virus (HCV)	Flaviviridae	1HEI	2	451	2.10	27–118
SARS-CoV-2	Coronaviridae	7NIO	2	603	2.20	287–406 445–569

*obtained from MMDB database (https://www.ncbi.nlm.nih.gov/Structure/MMDB/mmdb.shtml)

Screening of the phytochemicals

The first level of screening for phytochemicals was performed based on prediction of drug-like properties. To achieve this, the drug like properties was obtained by using Lipinski's rule of five filter [26]. Compounds were selected based on whether they did not violate the rule.

Molecular Docking Study

Molecular docking is used to calculate the binding affinity between selected ligands and receptors, as well as the pose of the ligand on the receptor and the atomic interaction between them. It is a computational approach, and the program uses two types of algorithms, such as searching and scoring with a specific force field, to complete the docking task [27, 28]. In the present study, the Autodock Vina tool was used (https://vina.scripps.edu/) for molecular docking purposes. Autodock Vina is a preferred docking program of the researchers around the world. During the docking program setting, polar hydrogen and Kollman charges were added to the ligand using the ADT tool. Then both the ligand and receptors were converted to the PDBQT format. Further, the grid box was created around the receptor by selecting the *grid parameters* (dimension values in the x, y, and z axis on the receptor molecule). Autodock Vina uses Lamarckian Genetic Algorithm (LGA) as the searching algorithm in the molecular docking simulation process [29].

Toxicity risk assessment and ADMET study of ligand molecule

The toxicity potential of the selected ligands was then further analyzed by the OSIRIS Property Explorer server (https://www.organic-chemistry.org/prog/peo/). The OSIRIS Property Explorer software predicts various aspects of toxicity, such as high risks of undesired effects like mutagenicity or poor intestinal absorption, with a color-coded result. The ADMET analysis was also performed by the SwissADME server (http://www.swissadme.ch/). The specific ligand molecules were selected based on the above analysis, and the docking score.

Molecular dynamics simulation study

Molecular dynamics simulations of the best selected ligand-receptor complexes were performed in the aqueous environment. PRODRG server (http://davapc1.bioch.dundee.ac.uk) was used to obtain the Gromacs compatible topology of the ligand molecule. In the initial step of MD simulation, a cubic box was created around the protein-ligand complex, to which solvation was made by adding water molecules by choosing the Simple Point Charge (SPC) water model. Then the solvated system was neutralized by adding Sodium ion (Na^+) and chloride ion (Cl^-) having the ionic strength of 0.1M. GROMOS43a1 force field was selected for the simulation purpose. The MD simulations were executed using the GROMACS 5.1.1 package (https://www.gromacs.org/) installed on Ubuntu. The NPT ensemble was set at 300 K and a pressure of 1 bar. The system was energy minimized using 5,000 steps using the steepest descent methods. After the energy minimization NVT (number of particles, volume, and temperature) and NPT (number of particles, pressure, and temperature) equilibration was performed for 100 picoseconds (ps). The simulation was conducted at 50 nanoseconds time steps. Then after simulation different parameters was analysed the trajectory such as Radius of Gyration (Rg), Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), H-bond interactions. Further, Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) calculations of binding free energy calculation was performed on the Gromacs platform using the *g_mmpbsa* package [30]. The tool was used to calculate the binding free energy for the ligand-receptor complexes for which it implements the Molecular Mechanic/Poisson-Boltzmann Surface Area (MM-PBSA) algorithm [31, 32].

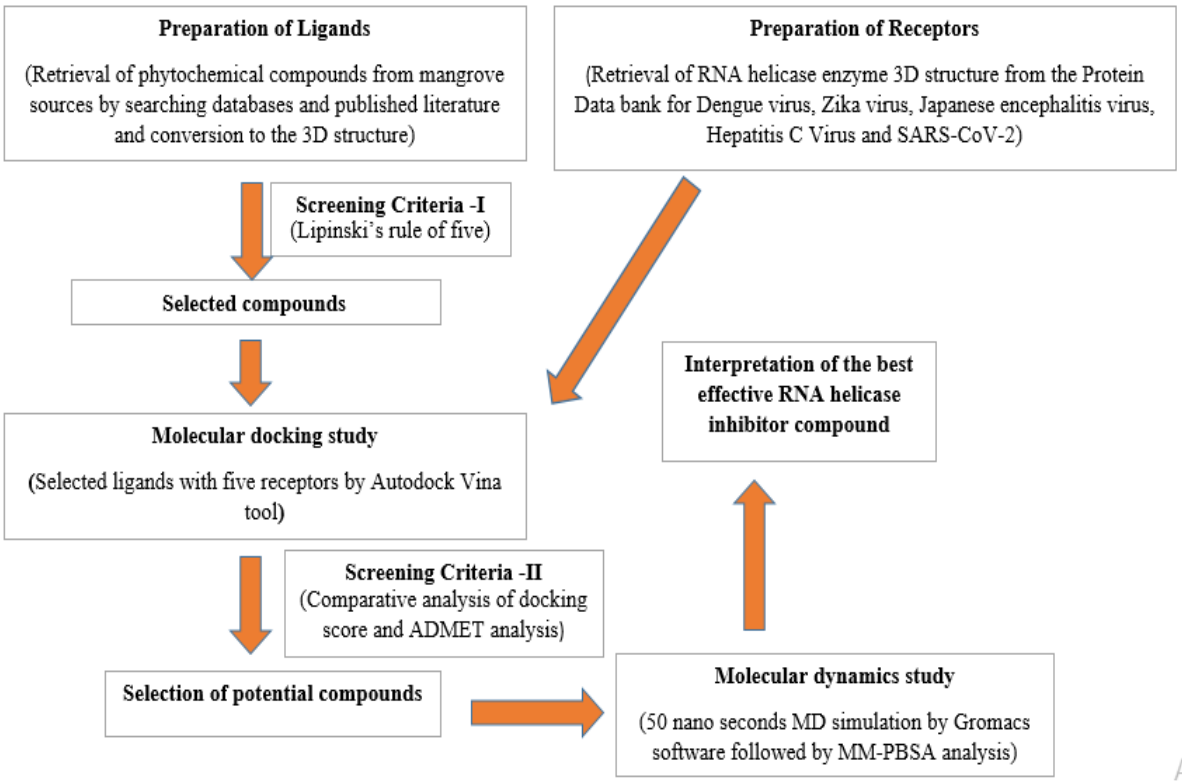

Fig. 1. Schematic presentation of the methods used in the present work.

RESULTS

Screening and molecular docking study

A total of 59 mangrove phytochemicals were obtained from the search, and 19 numbers of compounds were selected based on Lipinski's five rules (Supplementary material).

All 19 ligands were docked to five types of RNA helicase receptors (Table 2). After screening based on docking score, and toxicity, the molecule gedunin showed the best result. Further toxicity screening was performed by using the OSIRIS tool, followed by SwissADME server for other pharmacological property (Table 2).

From the above analysis, it was observed that, the phytochemical gedunin from the mangrove plant *Xylocarpus granatum* is showing the overall good docking result with all the selected five receptors (bold in Table 2).Similarly, the ADMET properties of the compound were obtained satisfactory (bold in Table 3) in comparison to the other selected ligands.

Further, the gedunin interaction profile with the selected receptors were analysed and shown in Figure 2. Gedunin formed three H bonds with amino acids ARG A: 599, LYS A: 388, and ALA A: 292 against the 2BMF receptor. Two H bond with amino acids SER A: 231, THR A: 212 against 1HEI, two H bond with amino acids SER A: 293, ARG A: 388 against 5K8U, two H bond with amino acids HIS A: 488, ARG A: 600 against 2Z83 and two H bond with amino acids ASN A: 177, ASN A: 179 against 7NIO. However, in all cases, no ATP binding residues of the receptors were observed to be involved with the gedunin (ligand) interaction.

Table 2. Docking score off the selected phytochemicals with five different viral helicase enzymes

S.N	Ligand Name	PubChem CID	Receptors and docking score (Kcal/mol)				
			1HEI	2BMF	2Z83	5K8U	7NIO
1	2-benzoxazolinone	6043	–5.4	–5.9	–5.9	–5.4	–5.9
2	7-tridecanone	10015	–4.5	–3.7	–4.2	–4.3	–5.9
3	Acanthicifoline	442503	–5.3	–5.8	–6.3	–6.1	–5.6
4	Arabinopyranoside	439195	–5.1	–5.4	–5.9	–5.9	–5.4
5	Benzoic Acid	243	–5.5	–5.3	–5.5	–5.2	–5.4
6	Benzopyran	9211	–5.5	–5.3	–5.2	–5.5	–5.3
7	Bruguisulferol	11513780	–5.5	–4.5	–4.6	–4.5	–4.6
8	Capsacin	1548943	–6.2	–6.7	–5.9	–6	–6.6
9	Coumarin	323	–6.2	–6	–6	–6.2	–6
10	Decanoic Acid	2969	–5.2	–4.3	–4.4	–5	–4.8
11	Diethylhydroxylamine	19463	–3.8	–3.9	–3.7	–4.1	–3.5
12	**Gedunin**	**12004512**	**–9.2**	**–8.9**	**–8.2**	**–9.7**	**–8.8**
13	Hygroline	270601	–4.8	–5.1	–4.4	–4.6	–4.8
14	Lapachol	3884	–7.4	–7.8	–6.8	–7.8	–7.2
15	Levoglucosan	2724705	–5.2	–5.8	–5.7	–5.5	–5.4
16	Lignan	261166	–6.8	–6.4	–6.3	–7.3	–7.2
17	Pentanoic Acid	7991	–4.3	–4.2	–4.4	–4	–4.2
18	Quinizarin	6688	–7.3	–7.7	–4.4	–7.9	–7.2
19	Xanthone	7020	–6.5	–6.9	–6.8	–7.5	–7.1

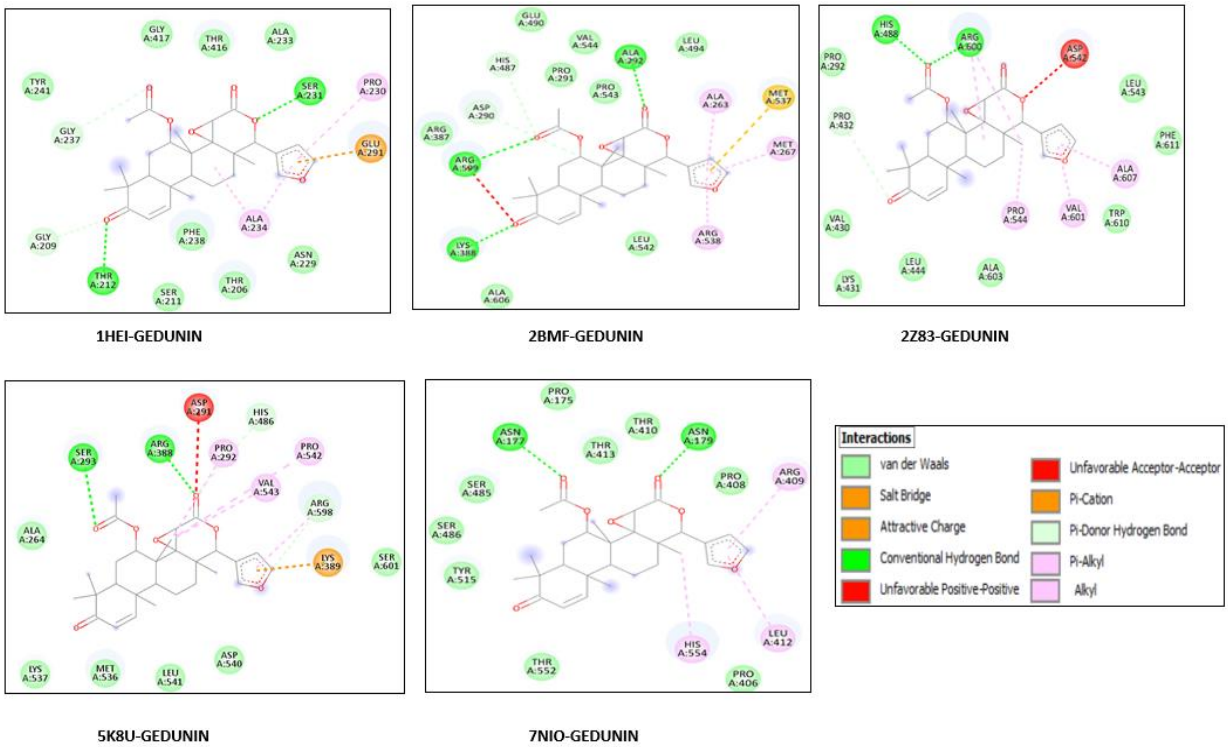

Fig. 2. Interaction of gedunin with five different viral RNA helicase enzymes (pdb IDs corresponds to different virus RNA helicase enzyme as given in Table 1).

Table 3. ADMET property of the selected ligand molecules computed from the SwissADME server

S.N	Compound	GI abs	BBB	Bioavailability Score	Log Kp	Synthetic Accessibility
1	2-benzoxazolinone	High	Yes	0.55	–6.3	2.34
2	7-tridecanone	High	Yes	0.55	–4.07	1.93
3	acanthicifolin	High	Yes	0.55	–7.47	2.59
4	Arabinopyranoside	Low	No	0.55	–9.36	3.8
5	Benzoic acid	High	Yes	0.85	–5.72	1
6	Benzopyran	High	Yes	0.55	–5.51	2.55
7	Bruguisulferol	High	No	0.55	–7.81	3.08
8	Capsacin	High	Yes	0.55	–5.62	2.32
9	Coumarin	High	Yes	0.55	–6.2	2.74
10	Decanoic acid	High	Yes	0.85	–4.45	1.67
11	Diethylhydroxylamine	High	Yes	0.55	–6.48	1
12	**Gedunin**	**High**	**No**	**0.55**	**–6.25**	**6.48**
13	Hygroline	High	No	0.55	–6.52	2.15
14	Lapachol	High	Yes	0.85	–5.8	2.98
15	Levoglucosan	High	No	0.55	–8.82	4.87
16	Lignan	High	No	0.55	–6.42	4.66
17	Pentanoic acid	High	Yes	0.85	–5.94	1
18	Quinizarin	High	Yes	0.55	–5.17	2.32
19	Xanthone	High	Yes	0.55	–5.09	2.76

Molecular dynamics simulation study

After 50 ns molecular dynamic simulation, the trajectory for all the five receptor –gedunin complexes were analyzed. The parameters such as RMSD is calculated by taking the average distance of complexes between atoms in the proteins during the MD simulation process [34]. In this work, the overall RMSD of the Cα atoms of the complexes were obtained as < 0.6 nm. In all cases, the ligand-receptor complexes show less deviation in comparison to their receptor counterpart. Considering all five simulations, the complex 5K8U-Gedunin showed the best result having a deviation < 0.35 nm (Figure 3).

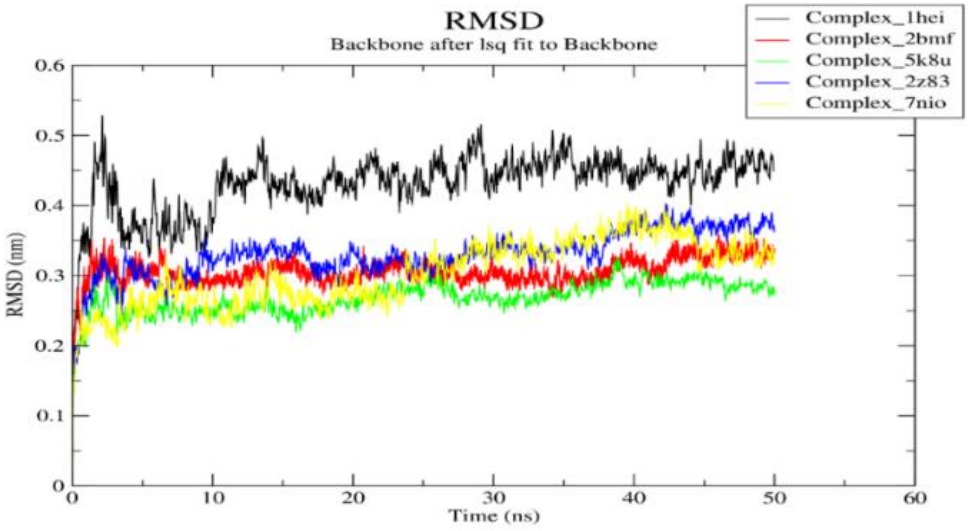

Fig. 3. RMSD plot of the gedunin-receptor complex.

The RMSF parameter calculates the protein's flexibility property and generates the individual amino acid flexibility over the simulation time [35]. RMSF provides the fluctuation of each atom in the overall simulation. RMSF was calculated for all the receptor-gedunin complexes, and overall fluctuations in all cases were obtained as < 0.7 nm. Little fluctuation differences in all protein-ligand systems indicate the stability in all complexes (Figure 4).

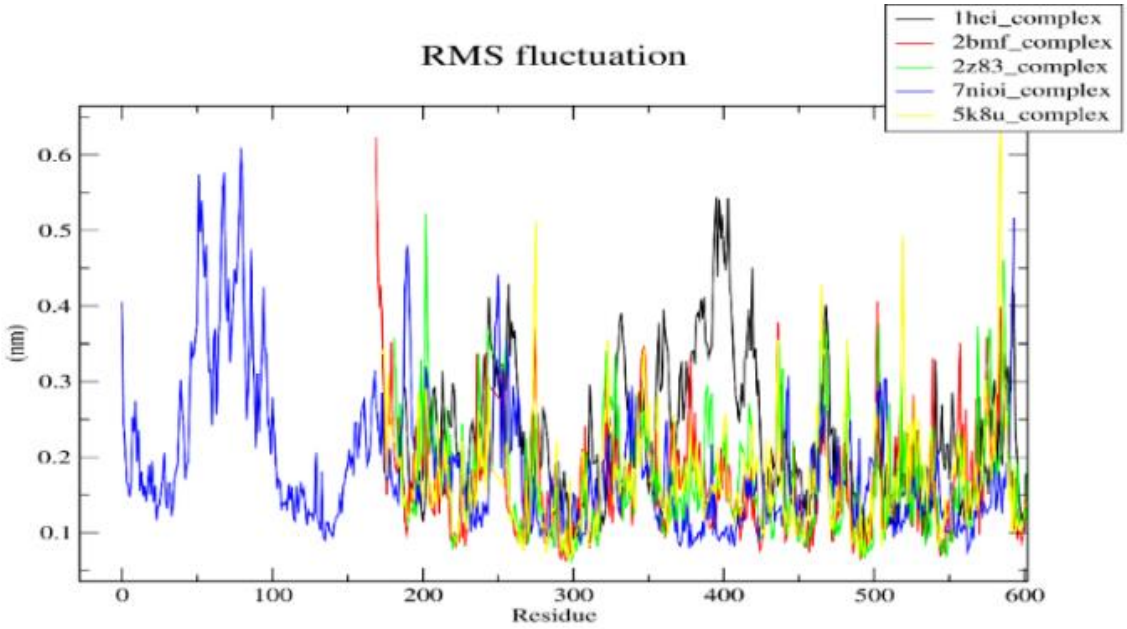

Fig. 4. RMSF plot of the gedunin-receptor complex.

The radius of gyration (Rg) is used to assess the compactness of the molecular design and stability over the molecular dynamics simulation period [36]. The radius of gyration (Rg) of the protein and ligand complexes was found to be between 2.77 and 2.18 nm initially. The Rg values of the viral RNA helicases with gedunin were stabilized after 10 ns and in the case of all. Also, the values were observed to occur in a decreasing trend initially and stabilized from 10–50 ns which is the indicator of stable binding of a ligand (Figure 5).

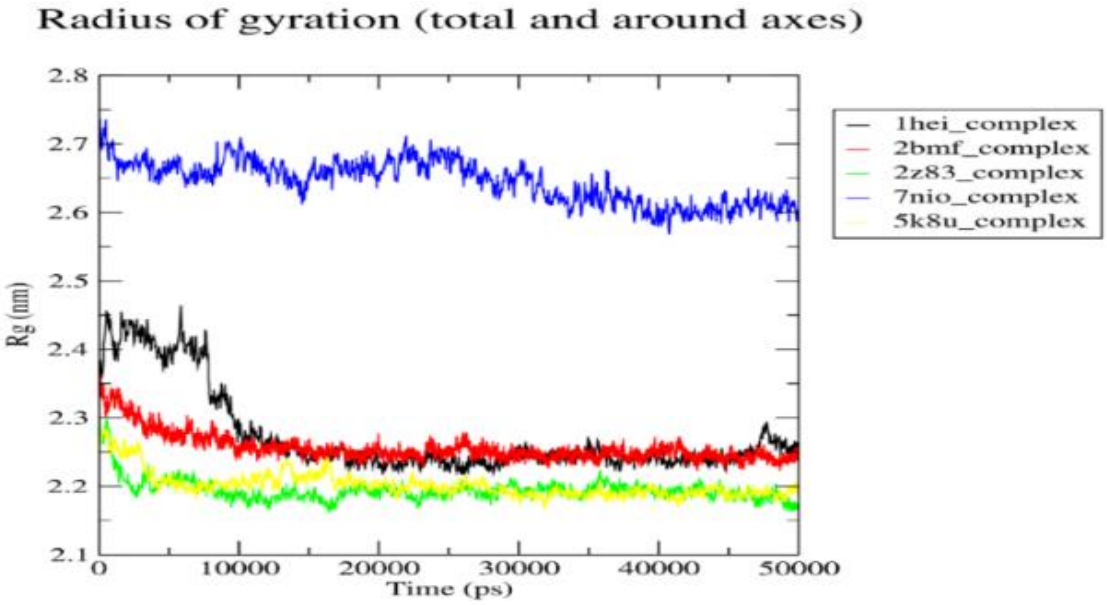

Fig. 5. Rg plot of the gedunin-receptor complex.

A protein's solvent-accessible surface area (SASA) property is calculated as the area accessed by the solvent (water). So analyzing the SASA value during MD simulation is crucial to identifying the conformational changes occurring during atoms' dynamics activity. The computed range of SASA values of five receptor-Gedunin complexes for 50 ns simulation was obtained as 23 nm^2 (1HEI-gedunin) between the 30 nm^2 (2BMF-gedunin), 32 nm^2 (2Z83-gedunin), 35 nm^2 (5K8U-gedunin), 36 nm^2 (7NIO-gedunin) respectively (Figure 6). These findings indicated that during 50 ns simulation, the SASA profile for the 1 HEI-gedunin complex was obtained as the lowest compared to the other four protein-ligand complexes, and for 7NIO-gedunin complex, it was obtained as the highest one.

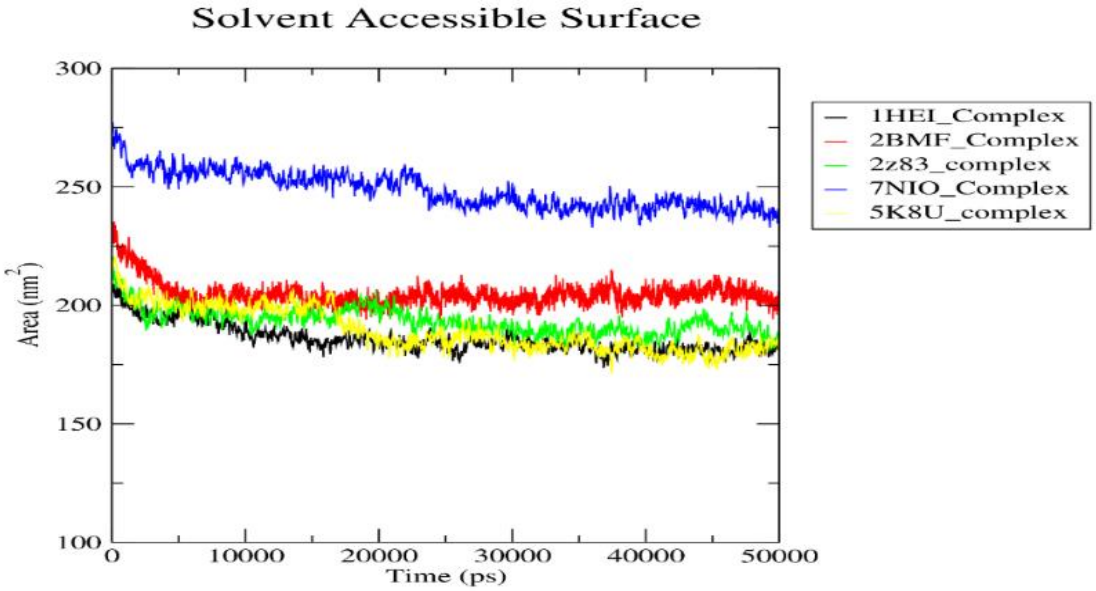

Fig. 6. SASA plot of the gedunin-receptor complex.

Hydrogen bond analysis

Understanding atomic level interaction is very important to predict the hydrogen bonding profile of the ligand with the receptor during the MD simulation.

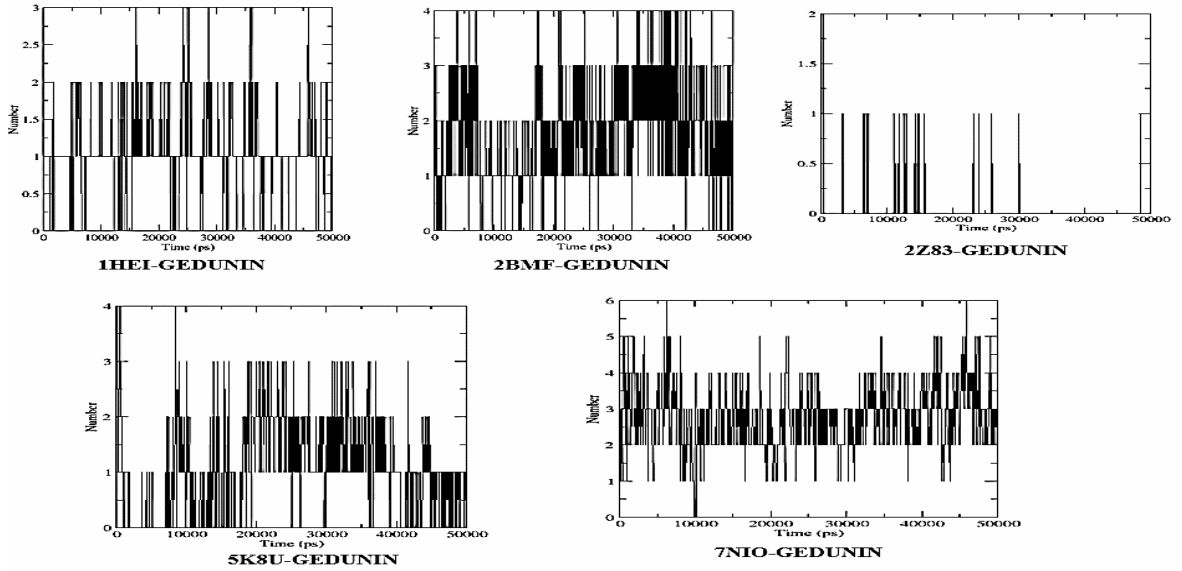

Fig. 7. Hydrogen bond formation trend in the gedunin-receptor complex.

Therefore, the intermolecular interactions, such as hydrogen bonds, were investigated over 50 ns of MD simulation studies. The study stated that the ligand geduin – 7NIO complex was able to form maximum numbers of hydrogen bonding (9), and the 2Z83-gedunin complex showed the lowest no of hydrogen bonding (1) during the simulation (Fig. 7).

MM-PBSA analysis

The binding energy of five docking complexes was calculated using the MM-PBSA method. The results in Table 4 indicate the estimation of MM/PBSA in kJ/mol. In addition, for each complex, different energy terms such as Vander Wall, Electrostatic, Polar salvation, and SASA energy were computed (Table 4).

Table 4. Showing the MM-PBSA calculation for the RNA helicase-gedunin complexes after MD simulation

Energy Terms (kJ/mol)	1HEI-gedunin	2BMF-gedunin	2Z83-gedunin	5K8U-gedunin	7NIO-gedunin
Van der Waals energy	–181.887 ± 30.396	–215.251 ± 46.924	–212.513 ± 11.854	–216.558 ± 70.043	–254.389 ± 61.496
Electrostatic energy	–77.233 ± 21.877	–77.715 ± 27.323	–22.088 ± 16.309	–38.148 ± 23.625	–109.500 ± 29.668
Polar solvation energy	155.192 ± 38.303	253.697 ± 64.171	116.696 ± 25.753	175.572 ± 69.342	220.792 ± 61.517
SASA energy	–16.424 ± 2.309	–17.789 ± 4.071	–16.022 ± 1.240	–16.164 ± 6.062	–18.694 ± 5.022
Binding energy	–120.352 ± 21.537	–57.059 ± 22.066	–133.927 ± 17.243	–95.298 ± 40.731	–161.791 ± 35.885

1

Furthermore, there is a more significant contribution of Van der walls energy (–254.389 ± 61.496 kcal/mole), electrostatic contribution (–109.500 ± 29.668 kcal/mole), and SASA energy (–18.694 ± 5.022) for the 7NIO-geduninsystem than the other systems. Additionally, the polar solvation energy was found more contributed in the 2BMF-gedunin system (253.697 ± 64.171 kcal/mole). 7NIO complex showed the least binding energy, i.e., –161.791. For other complex such as 1HEI, 2BMF, 2Z83, and 5K8U computed binding energy were computed as –120.352, –57.059, –133.927, –95.298 kJ/mol respectively. The results of the molecular dynamics simulations and the binding energy estimation by the MM-PBSA method showed that the7NIO-gedunin molecular system is more stable, followed by 2Z83- gedunin and 1HEI-gedunin.

DISCUSSION

Recently, phytochemicals have become an essential therapeutic source targeting the viral RNA helicase enzyme, inhibiting viral replication. Due to the great diversity of these compounds, it was possible to use computational methods to find specific compounds as novel RNA helicase inhibitor molecules [37–39]. For example, the tetranortriterpenoids phytochemicals are reported to be potent antiviral compounds and have demonstrated their efficacy against several pathogenic viruses (DENV, Ebola virus and Coxsackie B virus, Ebola virus), etc. [40, 41]. Kumar, A. H. (2020) showed that the plant substance gedunin binds very effectively to the major protease enzymes of SARS-CoV-2 [42]. Various *in silico* methods, such as screening methods, molecular docking, and MD simulation techniques, are now widely used to facilitate the prediction of antiviral inhibitory potency of compounds. Molecular docking and MD simulation study have evaluated the effectiveness of gedunin, nimbolide, oxine acetate, and clactone with RNA polymerase enzyme of the Japanese encephalitis virus [43]. Kushwaha et al. analyzed the phytochemicals of the *Withania somnifera* plant compared

with the major protease enzyme of SARS-CoV-2. Molecular docking studies predicted that compounds such as quercetin-3-rutinoside-7-glucoside, rutin, and isochlorogenic acid would have high binding affinities compared to the enzyme's natural inhibitor molecules [44]. Reddy et al. studied the effect of crude plant extracts in inhibiting SARS-CoV-2 infection by targeting several viral proteins involved in various activities, such as viral host entry, polyprotein processing, and viral replication [45].

The key to using natural products as medicines is the analysis of compounds' toxic and other ADMET features to address the safety aspects. Concerning Gedunin, a published report by Braga et al. states that the toxic properties of the molecule are not well characterized in experimental and animal models [46]. Similarly, a previous experimental report stated that gedunin was observed to be relatively non-toxic to guinea pig cells [47]. Pharmacokinetic characterization of an ADMET study of gedunin in a mammalian model (rat) showed that the compound was associated with poor oral absorption and rapid blood clearance [48]. Tharmarajah studied the effects of gedunin on normal human cells by evaluating cell viability assays and found that gedunin administration did not exhibit cytotoxic properties [49]. Khalid et al. recently investigated the cytotoxic effects of gedunin on non-malignant cells and did not observe any inhibition. Furthermore, the compound gedunin did not exhibit chronic toxicity or other mutagenicity, hence it can be considered safe for therapeutic use [50].

CONCLUSION

The viral RNA helicase enzyme plays a vital role in the function of RNA genome replication and viral survival within the host, making it an important molecular target of choice. Mangrove plants produce various natural bioactive compounds with antiviral effects. Therefore, we investigated the RNA helicase inhibitor potential of several bioactive compounds from mangrove plants in this study. From 59 compounds, 19 molecules were selected based on screening for drug-like properties. These compounds were further studied based on molecular docking studies using the RNA helicase enzymes of five different pathogenic viruses. This was followed by ADMET studies in which the gedunin molecule was identified as having the most potential. Further molecular dynamics simulations (50 ns) and MM-PBSA analysis confirmed the above results. Gedunin showed high binding affinity and stability against SARS-CoV-2, followed by high binding affinity and stability against other viruses such as Japanese encephalitis and hepatitis C. Thus, the studied molecule gedunin can be used as a promising molecule for treating pathogenic viral infections. In addition, the potential derivatives of the compound can be designed and further investigated by specific experimental studies. Therefore, the studied molecule gedunin could be used as a promising molecule for the treatment of pathogenic viral infections. We can also design potential derivatives of compounds and explore them further through specific experimental studies.

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