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REVIEW ARTICLE

An *in silico* approach to evaluate the bindings of natural flavonoids and RNA–DNA hybrids

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ABSTRACT

Flavonoids, low molecular weight polyphenolic compounds, are important natural products that belong to plant secondary metabolites. They have diverse biomedical applications such as antioxidative, anti-inflammatory, enzyme inhibitory, antimutagenic, anticarcinogenic, aromatase inhibitory effects, etc. Some of the flavonoids have been exported for bindings with certain DNA and tRNA structures both experimentally and computationally. RNA–DNA hybrid (RDH) falls into an important category of noncanonical nucleic acid structures that have many important biological functions. We have investigated the interaction of RDH structures with some of the dietary flavonoids with the aid of computational methods such as docking and molecular dynamics simulation. The presence of the – OH group on the ligand and the availability of a proper binding pocket in the macromolecule are the two main factors driving the binding preference. Thus, this computationally guided report explains the binding of the flavonoids with RDH structures to assist the researchers in designing noncanonical nucleic acid-targeted drug molecules.

Abbreviations: RDH: RNA–DNA Hybrid; MD: molecular dynamics; RMSD: root mean square deviation; GAFF: general AMBER force field; ns: nanosecond; ps: picosecond; fs: femtosecond; PME: particle mesh Ewald; MM_GBSA: [molecular mechanics (MM), generalized Born (GB), surface area]; VDW: van der Waals

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KEYWORDS
Flavonoids; nucleic acid structures; RNA-DNA hybrid; MD simulation

Introduction

Flavonoids, widely found in natural sources like various fruits and vegetables, have wide applications in biomedical sciences (Kaviarasi et al., 2019; Wen et al., 2021). Among them, luteolin, apigenin, quercetin, and genistein are four natural plant-derived dietary flavonoids that have attracted the scientific community due to their great abundance and interesting chemical properties to explore in broad research applications (Adel et al., 2022; Lin et al., 2008; Yang et al., 2020; Zhang& Wu, 2022). These secondary metabolites of plants can also act as antioxidants, protecting cells from free radical damage (Pandey & Rizvi, 2009). There are reports which also prove the anticancer properties of flavonoids (Kopustinskiene et al., 2020).

Different nucleic acid structures (DNA, RNA, RNA–DNA hybrid, secondary noncanonical DNA/RNA conformations) are well-known targets of anticancer agents (Roy et al., 2020; 2021; 2022; Shaw & Arya, 2008; Tao et al., 2021; Tateishi-Karimata & Sugimoto, 2021). Small molecules which interact with these nucleic acid structures can alter their normal functions (Ali et al., 2016; 2017; Bhattacharya et al., 2010; Chaudhuri et al., 2021; Jain et al., 2009; 2012; Juru & Hargrove, 2021; Paul et al., 2012; Roy et al., 2022; 2023; 2023; Roy & Bhattacharya, 2022; Wheelhouse & Chaires, 2010). This is one of the most promising and well-accepted methods to achieve antiproliferative therapeutic goals. It has been reported that the anticancer properties of these dietary flavonoids *via* different pathways (Sepay et al., 2022; Shahrajabian et al., 2022). Very recently, Chen et al. (2020) published a report on the interaction of *Puerariae Radix* flavonoids and DNA that has been demonstrated both experimentally and computationally.

Numerous noncanonical nucleic acid structures are formed in the genome, which have important regulatory functions that have always been a topic of research. Among the noncanonical nucleic acid structures, RNA–DNA hybrids (RDH) have important functional properties, and the formation of such structures has been connected to a range of diseases (Brambati et al., 2020; Nadel et al., 2015; Ruth Stuckey et al., 2015). It has been observed that mutation of RNase H enzyme that distinctively hydrolyzes the RNA in RNA-DNA hybrids, RNA helicases, and topoisomerases is associated with the increased formation of RDH in the genome. Instead of embracing traditional B-conformation of DNA or A-conformation of RNA,

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RDHs adopt heteromerous or amalgam duplexes (Fedoroff et al., 1993). Thus, RNA-DNA hybrids, consisting of unique structures, supply the scope to utilize the structural difference for designing specific drug candidates.

Computational drug discovery helps researchers to find the potential lead compounds against any targets utilizing different computational tools. It may also be noted that though virtual screening plays an important part in the field of drug design, many false positive theoretical hits may also be obtained in this procedure (Cerón-Carrasco, 2022). To critically assess the pros and cons of the hits that are obtained from the virtual screening methods, subsequent refinements with experimental validations are necessary. Among many facets, the protonation state of the ligands, modification of the binding pockets of targets, and stability of the ligand–target interactions in the experimental condition can be determining factors for the validations in the *in vivo* condition. However, a preliminary molecular dynamics (MD) simulation should be executed to apprehend any prefatory issues.

Herein, four dietary natural flavonoids have been studied for the binding with RDHs by molecular docking, ADME properties, and MD simulations. *In silico* approach has been utilized to get structural insights into the bindings of flavonoids and RDH. Further, a literature-guided comparative study, determination of structure–activity relationship (SAR), and various simulation parameters were included to investigate the stabilities of the ligand–RDH complexes.

Result and discussion

Docking study. Here, we have used AutoDock Vina for docking purposes. However, it may also happen that the other score functions might outperform AutoDock Vina. During the last *ca.* 3 years, the docking method has been widely applied in the SARS-Cov-2 era, which led to the discovery of many virtual potential hits. In a recent report, the strengths and weaknesses of these approaches have been documented with the necessity of the support of complementary approaches, like MD simulation and experimental validations (Llanos et al., 2021). In this case, the most popular docking software turned out to be AutoDock Vina which has been used in 44.6% of the articles related to the main protease (Mpro) case study (Llanos et al., 2021).

Apigenin, genistein, luteolin, and quercetin (Figure 1) have been docked with four RNA-DNA hybrids (PDB: 479D, 1EFS, 1FIX, 1HG9) (Bondensgaard et al., 2000; Hantz et al., 2001; Horton & Finzel, 1996; Xiong & Sundaralingam, 2000). It has been observed that quercetin exhibited the highest binding energy with all the four macromolecules (Figures 2 and 3; Figure S1, Supporting Information). For 1FIX, luteolin and quercetin showed similar binding energies in the docking study (−7.4 kcal/mol). However, it has been noticed that apigenin and genistein showed less binding affinity as compared to quercetin and luteolin toward the four hybrid macromolecules. This can be attributed to the ortho-dihydroxy structure in the B ring of quercetin and luteolin.

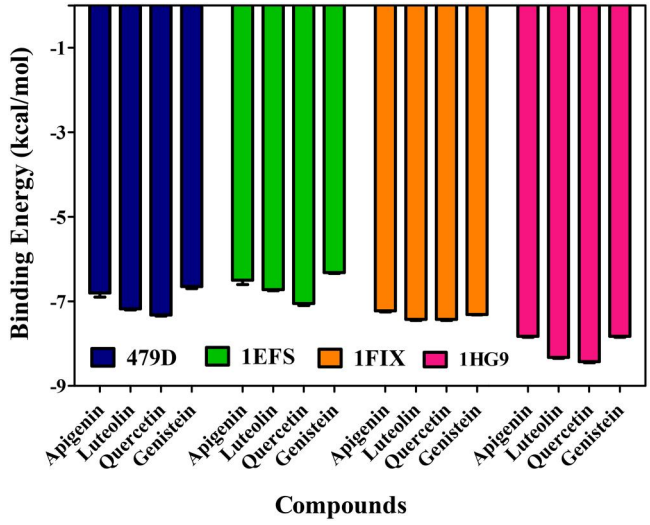

Figure 2. Comparative binding energies of the docked complexes.

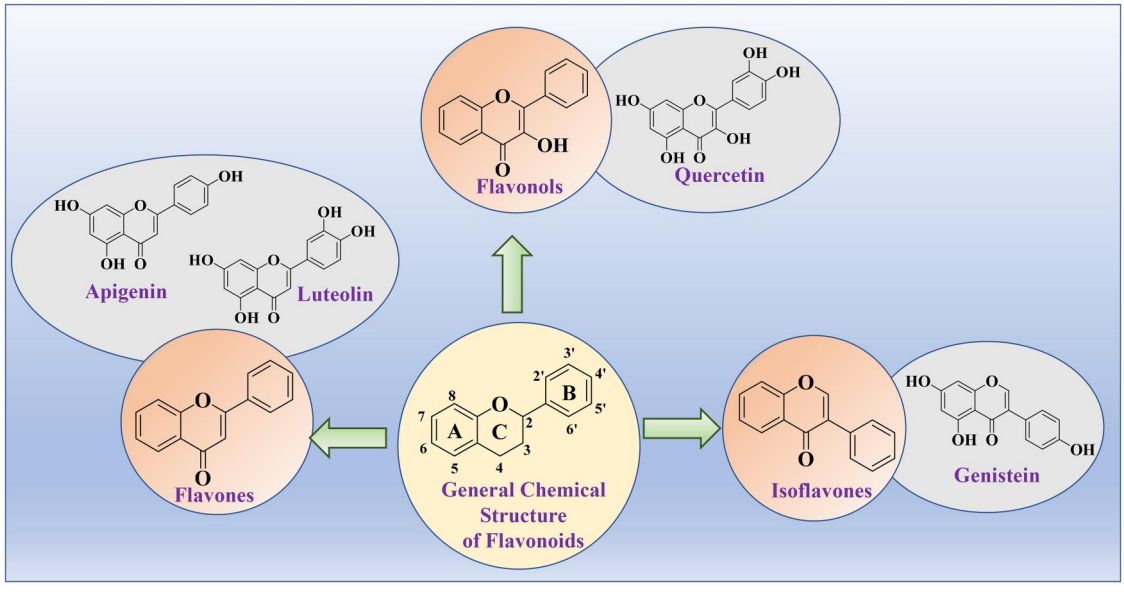

Figure 1. The chemical structure of flavonoids has been under-studied.

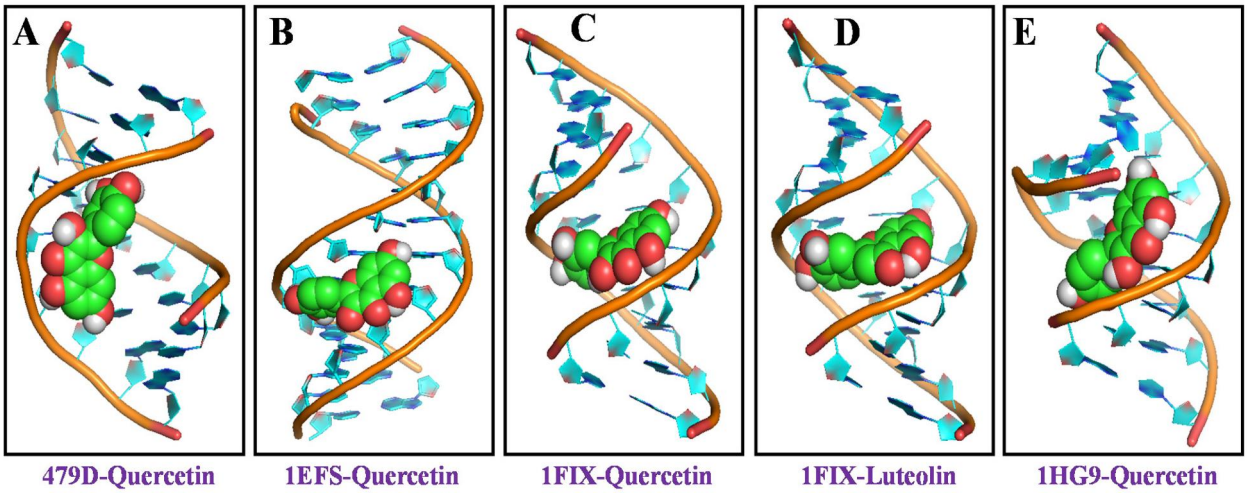

Figure 3. Docked poses of the RDH–ligand complexes. (A) 479D-Quercetin, (B) 1EFS-Quercetin, (C) 1FIX-Quercetin, (D) 1FIX-Luteolin, (E) 1HG9-Quercetin.

Table 1. ADMET profiles of the flavonoids.

Parameter	Apigenin	Luteolin	Quercetin	Genistein	Unit
Molecular properties					
Molecular weight	270.24	286.239	302.238	270.24	g/mol
LogP	2.5768	2.2824	1.988	2.5768	–
HB acceptors	5	6	7	5	–
HB donors	3	4	5	3	–
Absorption					
Water solubility	−3.329	−3.094	−2.925	−3.595	log mol/L
Human intestinal absorption	93.25	81.13	77.207	93.387	% Absorbed
Distribution					
VDss (human)	0.822	1.153	1.559	0.094	log L/kg
CNS permeability	−2.061	−2.251	−3.065	−2.048	log PS
Metabolism					
CYP3A4 substrate	No	No	No	No	–
CYP3A4 inhibitor	No	No	No	No	–
Excretion					
Total clearance	0.566	0.495	0.407	0.151	log ml/min/kg
Toxicity					
AMES toxicity	No	No	No	No	–
Max. tolerated dose (human)	0.328	0.499	0.499	0.478	log mg/kg/day
hERG I inhibitor	No	No	No	No	–
hERG II inhibitor	No	No	No	No	–
Acute toxicity (LD_{50})	2.45	2.455	2.471	2.268	mol/kg
Hepatotoxicity	No	No	No	No	–
Minnow toxicity	2.432	3.169	3.721	1.941	log mM

The structural differences in terms of the number and position of hydroxy groups in these polyphenol flavonoids contribute critically to their antioxidant activities (Zhang& Wu, 2022).

Pharmacokinetic profiling study. The pharmacokinetic properties of these four natural flavonoids have been investigated computationally. All four flavonoids obey Lipinski's rule, as observed in Table 1. A free online-available pkCSM tool was utilized to generate absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles (Pires et al., 2015). Apigenin and Genistein have exhibited a greater calculated human intestinal absorption profile as compared to Quercetin and Luteolin. Though greater volumes of distribution (VDss) values were observed for Quercetin and Luteolin as compared to Apigenin and Genistein. However, as these flavonoids have been established as anticancer agents, we have made an effort to support the experimental data with the calculated pharmacokinetic profiling data. It may also be noticed that the varying number of phenolic – OH in these flavonoids have an impact on their pharmacokinetic properties.

MD simulation study. In order to acquire molecular interactions between the ligands and macromolecule structures, an all-atom MD Simulation study has been performed (Figure S2, Supporting Information). Root mean square deviation (RMSD) plots indicate the stabilities of the ligands and RDHs during the simulation time periods (Figure 4). The fluctuations of RMSD are more in the case of 1EFS and 1FIX complexes as compared to 479D and 1HG9 complexes. Surprisingly, the relatively higher binding affinity of the 1HG9-Quercetin complex as evident from the docking study can be correlated with the less RMSD fluctuation as observed from the MD simulation study. This observation provides information on the greater stabilization of the 1HG9-Quercetin complex.

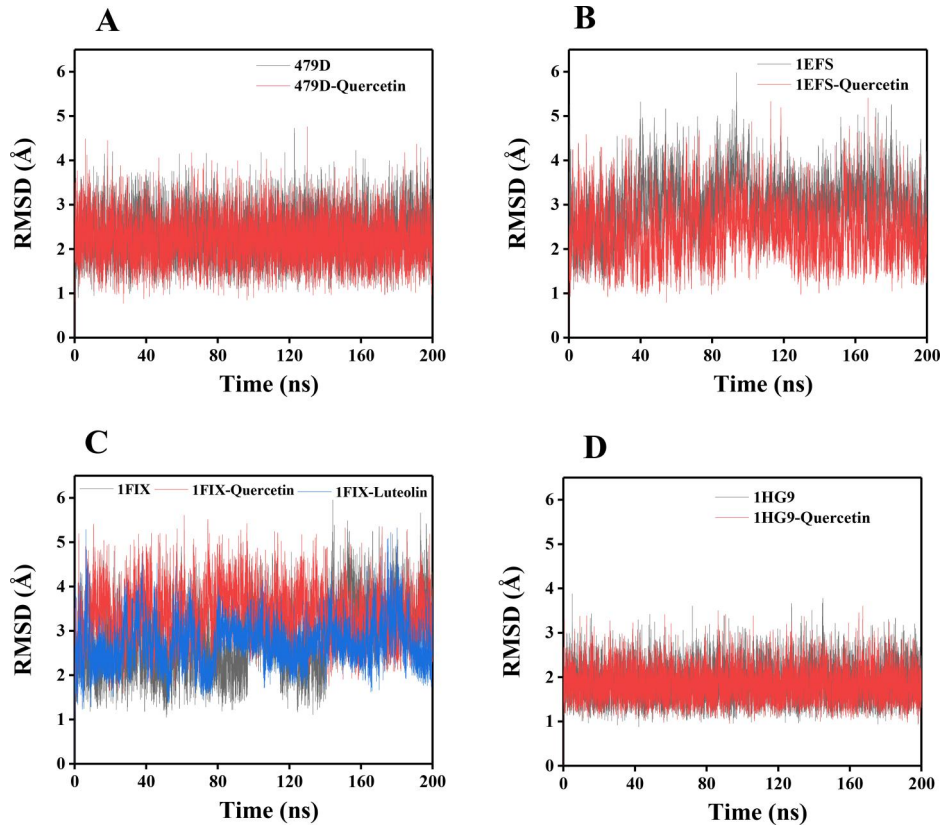

Figure 4. RMSD plots of the RDH and RDH–ligand complexes during 200 ns of MD simulation time period. (A) 479D and 479D-Quercetin; (B) 1EFS and 1EFS-Quercetin; (C) 1FIX, 1FIX-Quercetin and 1FIX-Luteolin; (D) 1HG9 and 1HG9-Quercetin.

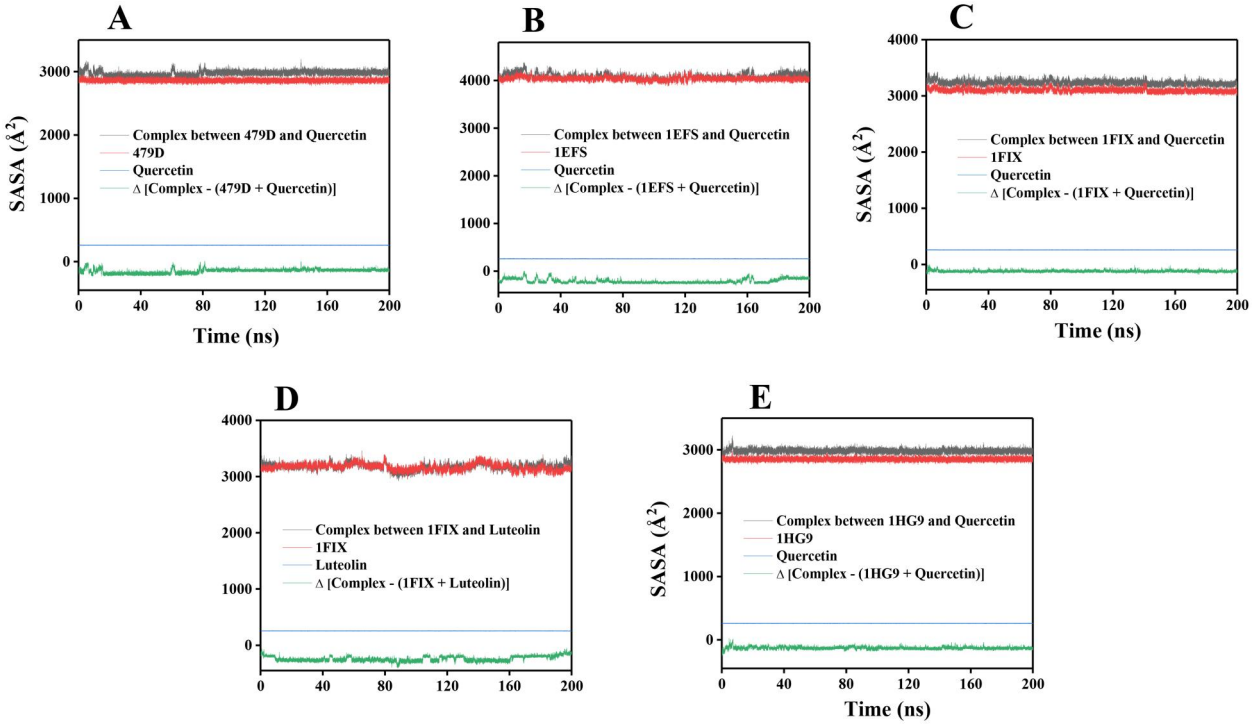

Figure 5. SASA plots of the complex, RDH, ligands.

Solvent accessible surface area (SASA). In computational studies, the solvent accessible surface area (SASA) parameter has always been a decisive factor to examine stability studies. SASA has been determined for the native RDH, RDH–ligand complexes, and both RDH and ligands extracted from the simulated complexes (Figure 5). It can be observed from Figure 5 that the ΔSASA [complex- (RDH + ligand)] value is negative throughout the simulation period. This is

an indication of the compactness of the RDH and ligand during the complexation. Further, a greater ΔSASA value of 1FIX-Luteolin is an indication of significant compactness of the complex that corroborates with the binding energy result.

Radius of gyration (Rg). From the calculated the radius of gyration values (Figure 6), it may be observed that the radius of gyration of the RDHs remains stable while interacting with the small molecules. It also indicates that the RDHs are compactly folded (Enayatkhani et al., 2022) and remain stable throughout the simulation period. This demonstrates the extent of compactness of the structures.

H-bonding analysis. Determining H-bonds between ligands and macromolecules is an influential factor in gaining the stability of the complex. To gain insight into this factor, we have analyzed the H-bond formations in the simulated complex (Table 2). The polyphenolic – OHs in the flavonoid structures provide an excellent platform for the H-bonding possibilities. The list of H-bonds has been given in Table S1, Supporting Information. However, a comparatively greater number of H-bonds have been observed in the case of the 1FIX-Luteolin complex probably because of the desirable fitting of the ligand in the binding pocket.

Free energy calculations. When free energies (MMPBSA and MMGBSA) were calculated for the five simulated complexes, the negative ΔG values specified the stabilities of all the complexes (Figure 7). However, 1FIX-Lut exhibited greater stability as observed from the ΔG value that further aligned with the docking result.

Apart from the free energy calculations, multiple replica molecular dynamics simulations, principal component analysis, and machine learning post-assessment may be applied to further refine the computational models (Liang et al., 2022). These methods decipher further information regarding the binding mechanism of the small molecule with the macromolecular targets. Further, the conformational changes, relative orientation, geometric positions, and movement pattern of the macromolecule and small molecules provide critical information regarding the binding mechanism and the prospective scope for the modification of the binding landscapes.

SAR of the four flavonoids toward the binding of RNA-DNA hybrids. The binding of the flavonoids and RNA-DNA hybrids may exhibit transcriptional regulation affecting the toxicities of the cells at different doses as has been observed earlier. Thus, a detailed structure–activity relationship is necessary not only for the molecular designing purpose but also to deeply understand how these small structural differences manifest powerful effects on several biological phenomena. The presence of phenolic – OH increases the possibilities of H-bond formations. The macromolecule structure plays an important role in the binding profiles. The structural difference between 1FIX and 1HG9 as compared to 479D and 1EFS in terms of available grooves formed by these RNA–DNA inter-strand can be attributed to

Table 2. H-bonding analysis of the complexes. (A) 1FIX-Quercetin. (B) 1FIX-Luteolin, (C) 1HG9-Quercetin.

(A).

Acceptor	DonorH	Donor	AvgDist (Å)	AvgAng (°)
DC_15@OP2	LIG_21@H1	LIG_21@O3	2.6285	166.0273
DC_15@OP2	LIG_21@H	LIG_21@O2	2.6265	166.0454
DG_14@OP2	LIG_21@H	LIG_21@O2	2.7049	160.8266
G_5@O6	LIG_21@H4	LIG_21@O6	2.7564	158.3867

(B).

Acceptor	DonorH	Donor	AvgDist (Å)	AvgAng (°)
DG_12@OP2	LIG_21@H3	LIG_21@O5	2.6517	165.0713
DG_14@OP2	LIG_21@H	LIG_21@O2	2.7188	165.8525
DC_13@OP2	LIG_21@H2	LIG_21@O4	2.6387	157.7168
DC_15@OP2	LIG_21@H	LIG_21@O2	2.6936	165.1675
DC_15@OP2	LIG_21@H1	LIG_21@O3	2.7222	152.6047
DC_13@OP2	LIG_21@H3	LIG_21@O5	2.6646	164.8205

(C)

Acceptor	DonorH	Donor	AvgDist (Å)	AvgAng (°)
G3_18@OP2	LIG_19@H	LIG_19@O2	2.6731	162.1790
A_12@OP2	LIG_19@H2	LIG_19@O4	2.6777	153.2762
A_14@N7	LIG_19@H4	LIG_19@O6	2.7970	156.7662
C_11@OP2	LIG_19@H	LIG_19@O2	2.6690	163.6934
G3_18@OP2	LIG_19@H4	LIG_19@O6	2.6503	163.2848
G3_18@OP2	LIG_19@H1	LIG_19@O3	2.7034	163.2445

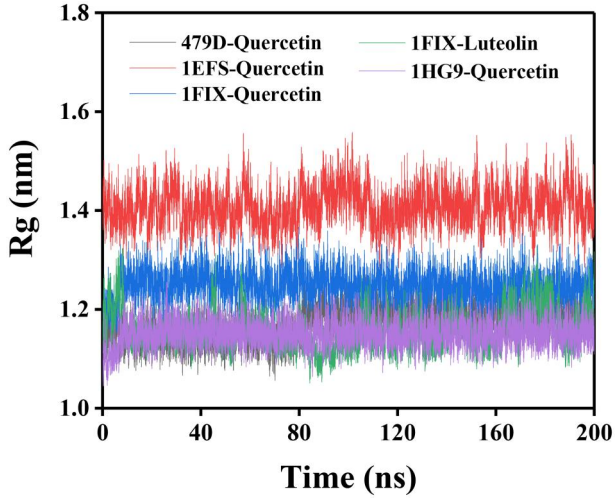

Figure 6. The radius of gyration (Rg) values of RDH-ligand complexes during 200 ns of production runs.

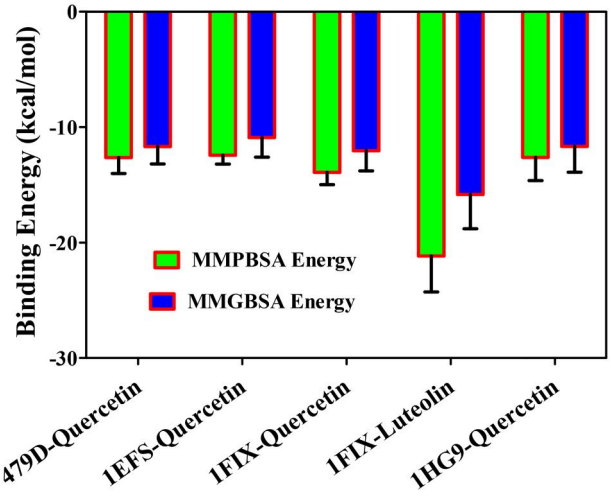

Figure 7. MMPBSA and MMGBSA free energies of the complexes.

the difference in binding energies. The greater binding energies of all four flavonoids with 1FIX and 1HG9 as compared to 479D and 1EFS have been possible due to the availability of proper binding pockets. Overall, the targets and the ligands should be properly examined to achieve a structure-guided drug design approach.

Comparison with reported experimental data. Flavonoids have been explored with multifaceted therapeutic applications (Atrahimovich et al., 2021). Flavonoids are reported to interact with DNA to exhibit anticancer properties (Kanakis et al., 2005). Metal flavonoid complexes have been presented to interact with DNA through electrostatic and intercalation modes of binding (Jabeen et al., 2019). The bindings of ten flavonoid derivatives with duplex DNA were analyzed by electrospray ionization mass spectrometric (ESI-MS) method deciphered that the 4′-OH group of flavonoid aglycones was necessary to manifest their DNA-binding properties (Wang et al., 2008). Tawani & Kumar (2015) investigated the interaction of flavonoids with non-canonical G-quadruplex structures. Quercetin was found as a potential candidate to bind with telomeric G-quadruplex DNA through intercalation mode. Very recently, Shukla et al. (2022) shed light on the interaction of flavonols with DNA through computational techniques as well as comparison with experimental data. The interaction of some flavonoids with DNA and tRNA has been examined along with their antioxidant properties. To the best of our knowledge, there has not been any effort to explore the interaction of RNA–DNA hybrids with these versatile flavonoids. This report will assist in getting the knowledge of molecular information for designing RDH-targeted drugs in the future.

Conclusion. These four flavonoids have been computationally studied for the binding of RNA–DNA hybrids. Binding interaction is dependent on the availability of the groove of RDH and the ligand structure. The number of – OH groups present in the ligands greatly influences the binding energies. The complexes have been probed for MD simulation studies. The stability of the complexes has been recognized by RMSD plots. Further, H-bonding analysis, SASA calculations, and free energy calculations guided the understanding of the stabilities of the complexes. A literature survey has been included to get information on DNA and tRNA-targeted flavonoids. Thus, this report consisting of RDH-targeted flavonoids enhances the structural insights toward computer-aided drug designing.

Methods

Initial structures. The structures of the ligands were obtained from PubChem with compound CID 5280443 for Apigenin, 5280961 Genistein, 5280445 for Luteolin, and 5280343 for Quercetin. Four RNADNA hybrid structures have been obtained from Protein Data Bank (PDB ID: 479D, 1EFS, 1FIX, 1HG9).

Docking. AutoDock vina was used for docking using Vina forcefield (Trott & Olson, 2010). The center of each RDH structure has been put as the center of the docking box with the box size setting to 126 Å in each dimension. The exhaustiveness of the global search was set to 8.

Molecular dynamics simulation. The association complexes obtained from docking studies were subjected to MD simulation studies. The simulation was performed using *Amber 18* software package along with *AmberTools 21* (Case et al., 2018, 2021). AM1-BCC method in the antechamber module was deployed to get the partial charges of the ligands utilizing the AMBER standard protocol. The other required force field parameters were procured from the AMBER GAFF (General AMBER Force Field) force field to be supported by LEaP (Table S2 and S3, Supporting Information). *parmchk2* was utilized to induce the other essential parameters along with the missing parameters. *tleap* module was employed for adding the H-atoms at the fixed positions of the macromolecules. The high negative electrostatic potentials around the RDHs were neutralized by adding K^+ ions. Explicit solvation was introduced by adding a 10 Å truncated octahedral shell of pre-equilibrated TIP3P water. Finally, the 200 ns simulations were carried out utilizing the *sander* module of *Amber 18* and the latest force field OL3 obtained from *AmberTools 21*. SHAKE algorithm was utilized for restraining the bonds that involve hydrogens. The necessary temperature of the system was endured with the assistance of the Langevin temperature equilibration scheme. The long-range electrostatics were assessed by using periodic boundary conditions which is generated on the particle mesh Ewald method. The RMSD was obtained by *ptraj* and the results were analyzed using Pymol and VMD.

Minimization. Energy minimization of the RDH and RDH-ligand complexes was performed in two stages. Initially, the positional restraints were applied with 500 kcal/mol force constants to maintain the rigidity of the RDH. Then, the second minimization stage was performed without any restraint.

Dynamics. The minimized structures were then heated from 0 to 300 K for a 20 ps simulation time period at constant volume with 50 kcal/mol restraining energy for RDH. After that, the equilibration was applied to the whole system. The temperature of the system has been controlled by Langevin dynamics with a collision frequency of 1.0 ps^{-1}. MD with 2 fs each time step has been carried out before the equilibration step. After that, the entire system was subjected to constant pressure. The constraints of the system were subsequently decreased (50, 40, 30, 20, 10 kcal/mol) for 50 ps each step for maintaining the equilibration at 300 K temperature. Then, a 1 ns final equilibration run with 5 kcal/mol minimal restraint was exerted to relax the system with ample time. Finally, the system was subjected to a 200 ns production run without any restraints.

H-bond analyses, Solvent Accessible Surface Area (SASA), Radius of Gyration (Rg), and Free Energy Calculation. H-bond analyses and SASA calculations were performed using the cpptraj utility of AMBER 21. The free energy difference calculation between the complexed and native RDH was carried out by employing the MM_GBSA [Molecular Mechanics (MM), Generalized Born (GB), Surface Area (SA)] and MM_PBSA [Molecular Mechanics (MM), Poisson-Boltzmann (PB) Surface Area (SA)] module of AMBER 21 (Zhang et al., 2017).

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