In silico screening of flavonoids targeting chromosomal replication initiator protein as a molecular target for new antibiotics in *Shigella dysenteriae*.

Cribado *in silico* de flavonoides dirigidos a la proteína iniciadora de la replicación cromosómica como diana molecular para nuevos antibióticos en *Shigella dysenteriae*.

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Highlights

- *In silico* screening identifies flavonoids targeting DnaA in multidrug-resistant *Shigella dysenteriae*.
- Corylin, a natural flavonoid, demonstrates high binding affinity to the DnaA protein in *Shigella dysenteriae* and shows potential as a lead compound for the development of new antibiotics.
- *In silico* ADMET predictions guide the selection of safe and effective drug candidates.

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ABSTRACT

Introduction. The bacterial chromosomal replication initiator protein, DnaA, plays an essential role in the bacterial cell cycle, making it a promising target for the development of new antibiotics. **Objective.** To identify flavonoids with potential to bind and inhibit DnaA through *in silico* screening. **Materials and Methods.** The DnaA sequence from *Shigella dysenteriae* was retrieved from the UniProt database. A homology model of DnaA was generated using SWISS-MODEL, a widely used and fully automated protein homology modeling server. Model accuracy was assessed using ERRAT, PROCHECK, and Molprobity. The refined model, obtained through the GalaxyWEB server, was then utilized in molecular docking experiments with a library of 300 flavonoids. The pharmacokinetic properties of the top-scoring compound, Corylin, were predicted using admetSAR 2.0. **Results.** Virtual screening of 300 flavonoids using AutoDock Vina software identified Corylin as a top-scoring ligand with a binding affinity superior to that of ADP. Molecular docking analysis revealed key interactions between Corylin and DnaA, including hydrogen bonds with residues T^{179}, R^{236} y R^{334}, as well as hydrophobic interactions with residues F^{141}, T^{174}, G^{175}, G^{177}, K^{178}, H^{180} e I^{305}. **Discussion.** Corylin is predicted to be absorbed in the gastrointestinal tract and does not appear to inhibit the Organic Cation Transporter 2 (OCT2). However, it may potentially inhibit the function of human cytochrome P450 enzymes. **Conclusions.** Preliminary *in silico* screening identifies Corylin as a potential lead compound for DnaA inhibition, warranting further investigation and validation *in vitro* and *in vivo* for its potential as an antimicrobial agent.

RESUMEN

Introducción. La proteína DnaA, iniciadora de la replicación cromosómica bacteriana, desempeña un papel fundamental en el ciclo celular, convirtiéndola en un objetivo prometedor para el desarrollo de nuevos antibióticos. **Objetivo.** Identificar flavonoides con potencial para unirse e inhibir DnaA mediante cribado *in silico*. **Materiales y Métodos.** La secuencia de DnaA de *Shigella dysenteriae* se obtuvo de la base de datos UniProt. Se generó un modelo de homología de DnaA utilizando SWISS-MODEL. La precisión del modelo se evaluó utilizando ERRAT, PROCHECK y Molprobity. El modelo refinado se utilizó en experimentos de acoplamiento molecular con una biblioteca de 300 flavonoides. Las propiedades farmacocinéticas se predijeron utilizando admetSAR 2.0. **Resultados.** El cribado virtual de 300 flavonoides identificó el flavonoide *Corylin* como un ligando de alta puntuación con una afinidad de unión superior a la del ADP. El análisis de acoplamiento molecular reveló interacciones clave entre el *Corylin* y el DnaA, incluyendo puentes de hidrógeno con los residuos T^{179}, R^{236} y R^{334}, así como interacciones hidrofóbicas con los residuos F^{141}, T^{174}, G^{175}, G^{177}, K^{178}, H^{180} e I^{305}. **Discusión.** Se predice que el *Corylin* se absorbe en el tracto gastrointestinal y no parece inhibir el Transportador de Cationes Orgánicos 2 (OCT2). Sin embargo, podría inhibir potencialmente la función de las enzimas del citocromo P450 humano. **Conclusiones.** El cribado *in silico* identifica al *Corylin* como un posible compuesto líder para la inhibición de DnaA, lo que justifica una mayor investigación y validación *in vitro* e *in vivo* por su potencial como agente antimicrobiano.

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INTRODUCTION

The World Health Organization has highlighted the global crisis of widespread bacterial resistance to antibiotics, prompting the development of innovative strategies for new antimicrobial discovery[1]. *Shigella* infections, a leading cause of mortality worldwide, pose a significant threat due to the emergence of multi-drug resistant strains, underscoring the urgent need for novel antibiotics with distinct molecular targets [1]. Flavonoids, a class of natural compounds found in plants, have shown promise in combating microbial infections caused by resistant organisms. For instance, flavones have demonstrated inhibitory activity against common wound pathogens in diabetic patients, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*[2, 3]. Furthermore, Zhang et al.,[4] suggested that Epigallocatechin Gallate (EGCG), a polyphenol found in tea, could potentially inhibit the growth of the gastrointestinal pathogen *Shigella flexneri*. These findings highlight the potential of phytochemicals as a valuable resource for the development of new antibiotics[5, 6].

Molecular docking, an in-silico approach widely used in drug discovery, enables the rapid screening of vast libraries of compounds to identify those that interact specifically with a target enzyme or protein[7]. For instance, Ye et al.,[8] employed QSAR and docking techniques to identify sigmacidins, benzoic acid-derived antimicrobial compounds capable of inhibiting streptococci by binding to the RNA polymerase σ factor. Similarly, Hosen et al.,[9] utilized iGEMDOCK software to identify glycoside derivatives with potential inhibitory activity against the YkuD L, D-transpeptidase protein (PDB ID: 4A1J) from *Bacillus subtilis*. Al-Khayyat[10] further demonstrated the utility of molecular docking by screening 50 DrugBank compounds against a kinase from the isoprenoid pathway in *Salmonella typhimurium* using AutoDock Vina. Additionally, Al-Khayyat[11] employed this approach to screen phytochemicals, including Amentoflavone, for their potential inhibition of chorismate synthase, a key enzyme in the shikimate pathway and a promising antimicrobial target.

Proteins involved in bacterial division machinery, such as the DNA replication initiator protein DnaA, represent promising targets for the development of novel antibiotics. While existing antibiotics like fluoroquinolones target type IIA topoisomerases[12], DnaA's dual function as an ATPase and transcriptional regulator, crucial for initiating chromosome replication at the oriC locus[13, 14], presents a unique therapeutic opportunity. This study investigates the potential of flavonoids as DnaA inhibitors in *Shigella dysenteriae*, with the aim of discovering new lead compounds for antimicrobial development."

MATERIALS AND METHODS

Sequence retrieval and binding site predictions

The sequence of the chromosomal replication initiator protein, DnaA (UniProt ID: Q329B6) from *Shigella dysenteriae*, was retrieved from the UniProt database (https://www.uniprot.org/uniprotkb). Binding site predictions were performed using the IntFOLD server (https://www.reading.ac.uk/bioinf/IntFOLD/)[15] to identify potential key amino acid residues involved in ligand binding.

Homology Modeling and Validation tests

A homology model of the DnaA protein was generated using SWISS-MODEL (https://swissmodel.expasy.org/)[16] without magnesium ions. The model was refined using the GalaxyWEB server (https://galaxy.seoklab.org/)[17], which optimizes the backbone and side chains by rebuilding loops and terminal segments. Model quality was assessed using the SAVES server (https://saves.mbi.ucla.edu/), specifically the ERRAT [18] and PROCHECK[19] tools, which evaluate structural errors and stereochemical quality, respectively. The

Molprobity server (http://molprobity.biochem.duke.edu/)[20] was also employed to further validate model accuracy through all-atom contact analysis.

Molecular Docking

The structure of ADP (ZINC12 ID: 12360703) was obtained from the ZINC12 database (https://zinc.docking.org/)[21] and used as a control ligand. A library of 300 flavonoids with molecular weights less than 500 Da[22] was curated from the Natural Product Activity and Species Source database (https://bidd.group/NPASS/browse_np_hierar.php). Flavonoid structures were converted to PDB format using Open Babel [23], and hydrogen atoms and Gasteiger charges were added. Non-polar hydrogens were merged to generate PDBQT files for docking. Molecular docking was performed using AutoDock Vina 1.1.2[24] with default parameters and a grid box of 60×60×60 Å centered at X=34.131, Y=83.056, and Z=13.796. Ten poses were generated for each ligand, and the resulting protein-ligand complexes were visualized using LIGPLOT[25] to analyze hydrogen bonding and hydrophobic interactions.

The top ten compounds with the highest binding affinities in AutoDock Vina were selected for re-docking using iGEMDOCK[26] with the following parameters: population size 200, generation 50, and run times 2.

Molecular dynamic simulation

Molecular dynamics (MD) simulations were performed using the SiBioLead online server (https://sibiolead.com/) following the method described by Chen et al.,[27]. The following parameters were used: OPLS/AA force field, simple point charge (SPC) water model, neutralization with NaCl at a concentration of 0.15 M, energy minimization using steepest descent for 5000 steps, temperature of 300 K, pressure of 1 bar (default), leap-frog integrator for 100 ns with 5000 frames.

Pharmacokinetics and toxicities

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the top ten ligands were predicted using the admetSAR 2.0 server (http://lmmd.ecust.edu.cn/admetsar2)[28].

RESULTS

Homology Modeling and Validation

A homology model of the DnaA protein from *Shigella dysenteriae* was generated using the SWISS-MODEL server[16], with 1l8q.1A (from *Aquifex aeolicus*) as a template (39.43% sequence identity) (**Figure 1**). The initial model was refined using the GalaxyWEB server[17]. Model quality assessment performed using ERRAT[18], PROCHECK[19], and Molprobity[20], showed significant improvement after refinement (**Table 1**), (**Figure 2**). The final model exhibited an ERRAT quality score of 94.753%, with 93.1% of residues in the most favored regions of the Ramachandran plot.

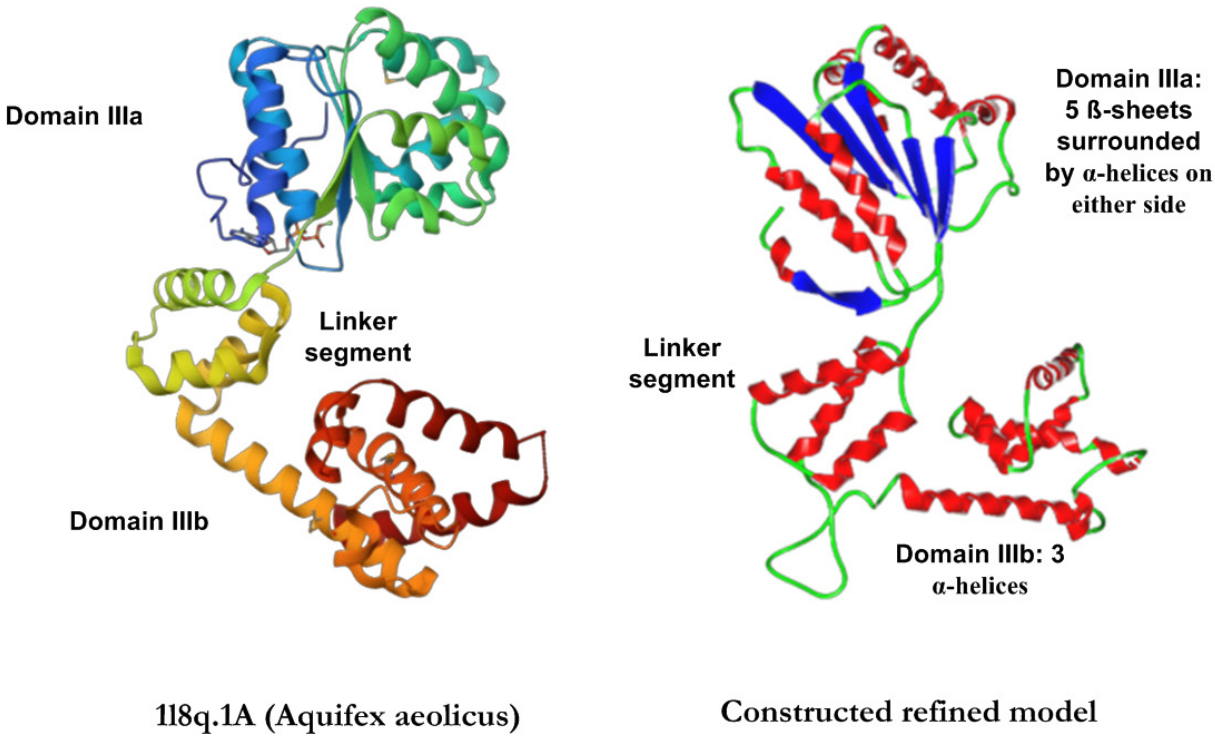
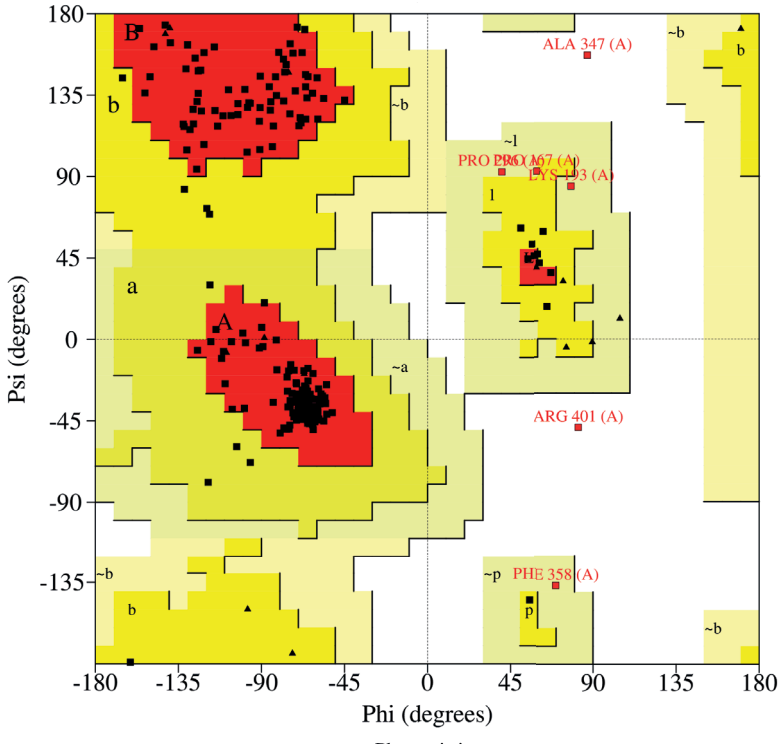


Figure 1. The built model of DnaA protein by SWISS-Model. Compared with the original template of *Aquifex aeolicus*.

Table 1: Protein evaluation by Molprobity server

Criterion	initial model (%)	Refined model (%)	Goal (%)
Poor rotamers	4.24	0.71	< 0.3
Favored rotamers	91.1	98.23	>98
Ramachandran outliers	2.7	0.90	<0.05
Ramachandran favored	91.59	96.40	>98
Rama distribution Z-score	-0.09 ± 0.45	0.78± 0.42	Abs Z-score <2.0
Cß deviation > 0.25 A°	3.16	3.48	0
Bad bonds	0.047	0.59	0
Bad angles	1.42	0.93	<0.1
Cis prolines	22.22	0.0	Expected ≤5%
Cis nonprolines	0.31	0.0	<0.05

Cß = C beta, Abs = Absolute



Plot statistics

Residues in most favoured regions [A,B,L]	284	93.1%
Residues in additional allowed regions [a,b,l,p]	17	5.6%
Residues in generously allowed regions [~a,~b,~l,~p]	2	0.7%
Residues in disallowed regions	2	0.7%
Number of non-glycine and non-proline residues	305	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	19	
Number of proline residues	9	
Total number of residues	335	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figure 2. Ramachandran plot as predicted by PROCHECK

Binding Site Prediction

The IntFOLD7 server predicted the following residues as potential participants in the DnaA binding site: N^{140}, F^{141}, V^{142}, N^{147}, G^{173}, T^{174}, G^{175}, L^{176}, G^{177}, K^{178}, T^{179}, H^{180}, D^{235}, I^{305}, K^{309}, V^{333}, R^{334}, E^{337}; Q^{215}, N^{217}, R^{245}; R^{399}, T^{435} (Figure 3).

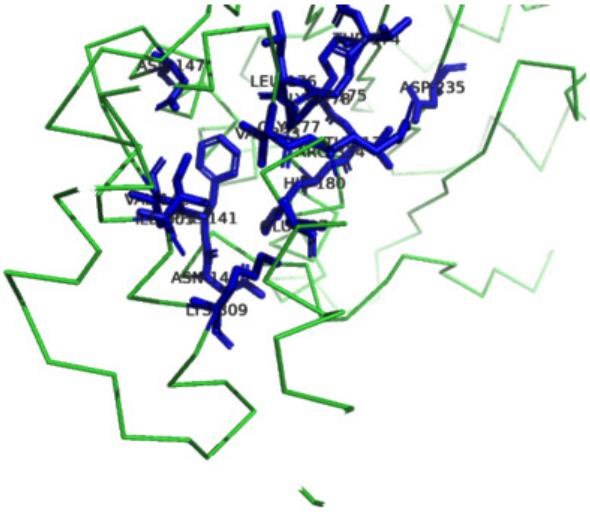


Figure 3. The binding pocket of DnaA protein as predicted by IntFold7 Molecular Docking.

Virtual screening of 300 flavonoids with molecular weights less than 500 Da (database available in the **Supplementary data, Table S1**) was performed using AutoDock Vina[24]. This screening, based on Lipinski's Rule of Five for drug-likeness[22], identified Corylin as the top-scoring ligand with a binding affinity of -9.1 kcal/mol, superior to that of ADP (-6.8 kcal/mol). Molecular docking analysis revealed key interactions between Corylin and DnaA, including hydrogen bonds with residues T^{179}, R^{236}, and R^{334}, and hydrophobic interactions with residues F^{141}, T^{174}, G^{175}, G^{177}, K^{178}, H^{180}, and I^{305} (**Figure 4**). The top ten ligands identified by AutoDock Vina were re-docked using iGEMDOCK (**Table 3**). 6-Cinnamylchrysin showed the highest docking fitness (-101.80 kcal/mol) with iGEMDOCK.

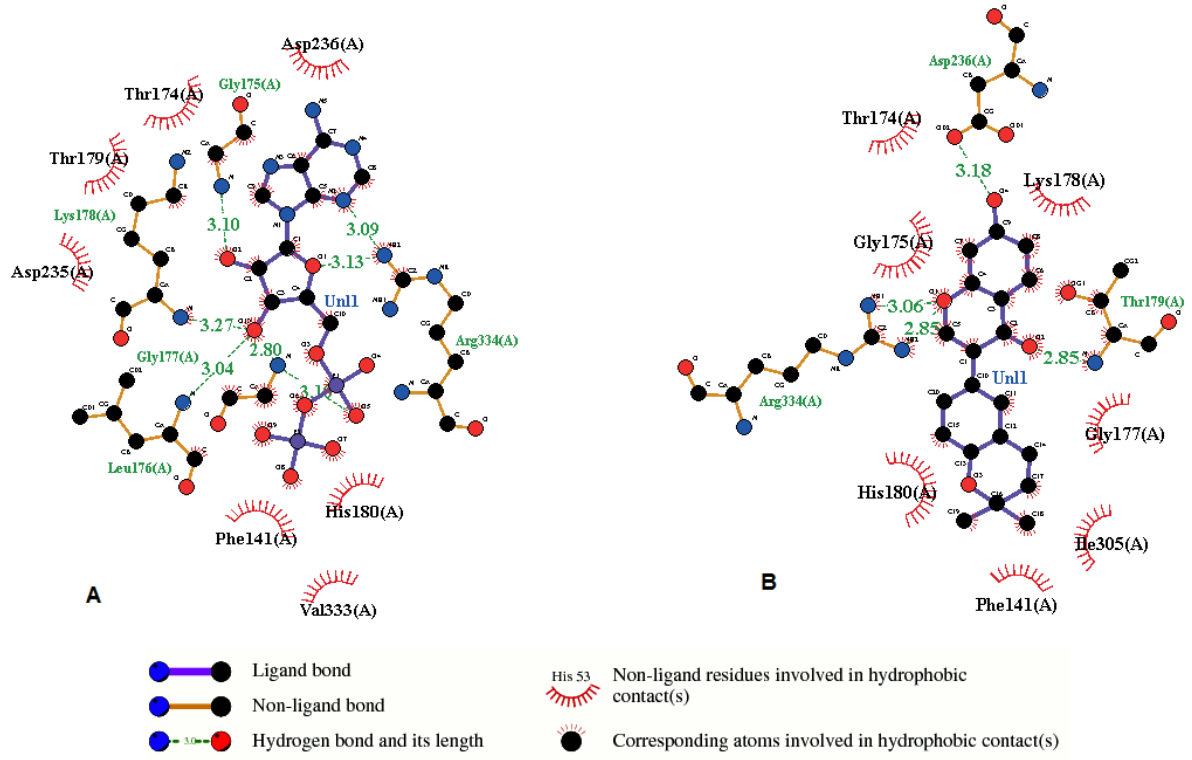


Figure 4. Interaction of ADP (A) and Corylin (B) with DnaA model as predicted by LigPlot

Table 3: Results of molecular docking by iGEMDOCK and interaction.

Compounds	Docking fitness (Kcal/mol)	Binding residues
ADP	-99.50	N^{140}, F^{141}, G^{175}, G^{177}, H^{180}, K^{308}, V^{333}, R^{334}
Corylin	-73.79	Y^{271}, K^{273}, D^{280}, K^{283}, G^{287}, T^{291}
Erythrabyssin I	-86.08	H^{136}, F^{141}, V^{142}, H^{182}, I^{305}
Buceracidin	-88.38	T^{174}, G^{175}, L^{176}, G^{177}, K^{178}, T^{179}, D^{235}, D^{236}, R^{334}
Licoflavone B	-85.13	T^{174}, G^{175}, L^{176}, G^{177}, K^{178}, T^{179}, H^{202}, R^{334}
Abyssinone I	-80.23	H^{136}, G^{175}, L^{176}, G^{177}, K^{178}, T^{179}, H^{180}, R^{334}
6-Hydroxy-8,8-dimethyl-2-phen-ylpyrano[2,3-h] chromen-4-one	-83.87	G^{175}, L^{176}, G^{177}, K^{178}, T^{179}, D^{236}, R^{334}
6-Cinnamylchrysin	-101.80	T^{174}, G^{175}, G^{177}, K^{178}, T^{179}, R^{334}
(S)-5,7,8"-trihydroxy-2",2"-dimeth-yl-2,6"-bichroman-4,4"-dione	-88.54	H^{136}, G^{177}, K^{178}, T^{179}, D^{235}, R^{334}
Meliternatin	-88.26	T^{174}, G^{175}, L^{176}, T^{179}, H^{180}, R^{334}
Artochamin C	-92.00	G^{175}, L^{176}, G^{177}, K^{178}, T^{179}, S^{331}, R^{334}

Molecular Dynamics Simulation

Molecular dynamics (MD) simulations of the Corylin-DnaA complex revealed that the system reached equilibrium after 25 ns, with a root mean square deviation (RMSD) not exceeding 0.8 nm (**Figure 5**). This indicates the stability of Corylin binding within the DnaA active site.

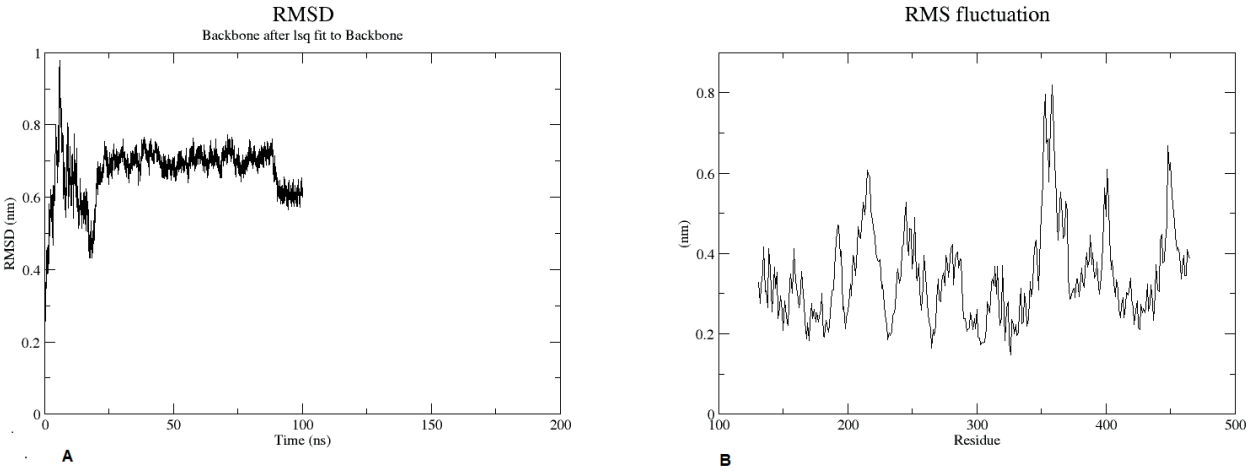


Figure 5. (A) Root means square deviation (RMSD) and (B) Root mean square fluctuation of Corylin-DnaA complex

ADMET Prediction

The ADMET properties of the top ten ligands were predicted using admetSAR 2.0 (**Table 4**). All ligands are predicted to be absorbed in the gastrointestinal tract. However, Erythrabyssin I and Meliternatin might penetrate the blood-brain barrier. Several ligands showed potential to inhibit cytochrome P450 enzymes and the hERG channel.

Table 4: Pharmacologic properties of the tested compounds represented by AdmetSAR 2.0 probabilities.

Compound	HIA **	BBB **	Inhibition of cytochromes					OCT2*	hERG**	Hepatotoxicity	Nephrotoxicity
			CYP3A4	CYP2C9	CYP2C19	CYP2C6	CYP1A2				
Corylin	+	-	+	+	+	-	-	-	-	-	+
Erythrabyssin I	+	+	-	-	+	-	+	-	-	-	-
Buceracidin	+	-	+	+	+	-	-	-	-	+	+
Licoflavone B	+	-	-	+	+	-	+	-	+	+	-
Abyssinone I	+	-	+	+	+	-	-	-	-	-	+
6-Hydroxy-8,8-dimethyl-2-phenylpyrano[2,3-h]chromen-4-one	+	-	+	+	+	-	-	-	-	-	-
6-Cinnamylchrysin	+	-	+	+	+	-	+	-	-	-	-
(S)-5,7,8”-trihydroxy-2”,2”-dimethyl-2,6”-bichroman-4,4”-dione	+	-	+	+	-	-	-	-	-	-	+
Meliternatin	+	+	+	+	+	+	-	-	+	+	-
Artochamin C	+	-	-	+	+	-	-	-	-	-	-

HIA = Human intestinal absorption, **BBB** = Blood brain penetration, **OCT2** = Organic cation transporters 2 inhibition, **hERG** = Human Ether-a-go-go-gene-Related gene inhibition.

DISCUSSION

The generation of a reliable 3D model of the DnaA protein from *Shigella dysenteriae* through homology modeling[29] validated by various assessment tools (ERRAT, PROCHECK, Molprobity)[18,19,20], represents a significant step towards the discovery of novel antibiotics[12]. The identification of Corylin as a top-scoring ligand in the virtual screening, corroborated by iGEMDOCK results suggests its potential as a DnaA inhibitor[26]. Molecular docking and dynamics simulations provide insights into the key interactions contributing to Corylin's binding affinity and stability within the DnaA active site[27, 30].

The selection of flavonoids with molecular weights less than 500 Da in this study was based on Lipinski's Rule of Five[22], a set of empirical criteria that predict the likelihood of a compound having favorable absorption and permeability properties. By adhering to this criterion, we aimed to prioritize flavonoids with higher potential for drug development, as compounds that violate Lipinski's Rule of Five often face challenges in oral bioavailability and membrane permeation.

It is important to note that homology modeling relies on the premise that proteins with substantial sequence similarity fold similarly, and the accuracy of the alignment between the target and template sequences significantly influences the accuracy of the model[29]. In this study, the 39.43% sequence identity between the DnaA protein of *S. dysenteriae* and the template from *Aquifex aeolicus* (1l8q.1A) justifies the application of this approach.

The three-dimensional structure of the DnaA protein, composed of three main domains (IIIa, IIIb, and IV)[31], reveals the importance of specific residues in ligand binding. The prediction of these residues using IntFOLD7[15] allowed for the identification of potential interaction sites for Corylin and other flavonoids. The validation of this homology model, which included an assessment of deviations from ideal bond lengths and angles, Ramachandran outliers, and clashing contacts[32], ensures the structural integrity and reliability of the model for further analysis. Additionally, the use of MolProbity for quality assessment provided updated dihedral-angle diagnostics and comprehensive all-atom contact analysis[33], further confirming the accuracy of the refined model.

The identification of Corylin as a promising lead compound is further supported by previous studies highlighting its therapeutic potential. Corylin, a natural product found in *Psoralea corylifolia L.,* has been shown to exhibit antioxidant, anti-tumor, and anti-inflammatory properties. Research by Hung et al.,[34] suggests that Corylin's anti-inflammatory action may suppress immunologic responses and potentially treat septic shock. Additionally, Zaidi et al.,[35] demonstrated the potent antimicrobial activity of *P. corylifolia L.,* extracts against *Helicobacter pylori*, with minimal bactericidal concentrations ranging from 15.6 to 62.5 µg/ml. These findings, combined with the results of our *in-silico* screening, reinforce the potential of Corylin as a valuable lead compound for antimicrobial drug development[36]. The use of a consensus scoring approach, employing both AutoDock Vina and iGEMDOCK, strengthens the reliability of our virtual screening results[37]. By comparing the scores from different docking programs with distinct scoring functions, we enhance the accuracy of ligand selection and improve the overall success rate of identifying potential inhibitors. The high docking fitness of 6-Cinnamylchrysin in iGEMDOCK (-101.80 kcal/mol) further supports its potential as a DnaA inhibitor.

However, the ADMET predictions[28] raise concerns about the potential for cytochrome P450 inhibition [38] and hERG channel interference[39] for some of the top-scoring ligands. These findings underscore the importance of further investigation and optimization to mitigate potential adverse effects. The inhibition of cytochrome P450 enzymes can lead to drug-drug interactions and affect the metabolism of other medications[39]. Similarly, hERG channel inhibition can cause cardiac arrhythmias and pose safety risks[38]. Additionally, the predicted blood-brain barrier penetration of Erythrabyssin I and Meliternatin[40] raises concerns about potential neurotoxicity, warranting further investigation into their safety profile. Moreover, while none of the compounds are predicted to inhibit OCT2, this transporter plays a crucial role in the renal clearance of many drugs, and its inhibition could lead to altered pharmacokinetics and potential drug interactions[41].

Overall, this study highlights Corylin and 6-Cinnamylchrysin as potential lead compounds for DnaA inhibition and warrants further exploration for their antimicrobial drug development potential[36, 42]. Future studies should focus on *in vitro* and *in vivo* validation of these findings, including assessment of their antimicrobial activity and potential toxicity. Additionally, the evaluation of the top-scoring ligands in animal models and clinical trials[43] will be crucial to determine their efficacy and safety as potential antimicrobial agents. The increasing prevalence of multi-drug-resistant microbial pathogens necessitates the exploration of alternative targets for new antibiotics, and DnaA, as an essential protein in bacterial DNA replication, presents a promising avenue for antimicrobial drug development[44, 45].

CONCLUSIONS

In this *in silico* study, we identified ten natural products with potential to bind and inhibit the bacterial chromosomal replication initiator protein, DnaA. Among these, Corylin, a natural flavonoid, exhibited the highest binding affinity compared to the native ligand ADP. Molecular docking and dynamics simulations suggest that Corylin forms stable interactions within the DnaA active site, highlighting its potential as a lead compound for antimicrobial drug development. However, ADMET predictions indicate a risk of drug interactions and potential toxicity due to cytochrome P450 inhibition and nephrotoxicity associated with some of the identified compounds, including Corylin. These findings underscore the need for further *in vitro* and *in vivo* pharmacological studies to validate the antimicrobial activity and safety profile of these natural products, particularly Corylin, before they can be considered for clinical development.

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