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In silico Study on Structural Inhibition of Bacterial DNA Gyrase by Major Secondary Metabolites Found in Grape Seed Extract

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Abstract

In the early 20th century, during the era of investigating and identifying essential "vitamins", scientific research focused on grape seed extracts and their bioactive components, particularly polyphenols. Extensive studies have demonstrated that grape seed extract, rich in proanthocyanidins, offers protection against a wide spectrum of diseases, encompassing inflammation, cardiac ailments, peptic ulcers, hypertension, diabetes, cancer, and microbial infections. To explore potential secondary metabolites within grape seed extract that could serve as structural inhibitors of bacterial DNA Gyrase, molecular docking studies were performed. The docking results revealed that two phytochemicals, namely (-)-catechin and Procyanidin-B2, exhibited the highest potency in inhibiting DNA gyrase subunit B. Subsequent in silico physicochemical and pharmacokinetic parameter predictions were conducted using specialized web servers for the examined phytochemicals. Notably, (-)-catechin displayed superior inhibitory and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics, suggesting its potential for utilization in synthesizing novel antibacterial compounds.

Keywords: Grape seed extract; Polyphenols; catechin; molecular Docking; Pharmacokinetic; Physicochemical.

INTRODUCTION

In recent years, extract from grape seed has grown in popularity as a dietary supplement, particularly in Australia, Korea, Japan, and the United States (Yamakoshi et al., 2002). This is due to the fact that grape seeds are high in phenolic compounds and may benefit human health in ways like preventing gastric ulcers (Kim et al., 2013; Rodríguez Montealegre et al., 2006) Grapevine seeds have a high antioxidant capacity; as a result, they can reduce the activity of antioxidant enzymes, protect cells from oxidative damage, have anti-inflammatory and anti-atherosclerotic properties, and prevent certain cancers in both humans and animals (Melov, 2002). Based on promising reports of their antioxidant capabilities and capacity to act as free radical scavengers, the polyphenols and flavonoids found in the Grape seed extract have attracted great attention (Georgiev et al., 2014).

The hunt for novel medications in the area of secondary plant metabolites as compounds for curing civilizational disorders has recently been the focus of pharmaceutical and commercial trends. The grapevine (*Vitis Vinifera L.*) seed's matrix contains significant physiologically active components that allow for its application in medicine (Sochorova et al., 2020) Proanthocyanidins and catechins are two types of flavan-3-ol that are abundant in grape seeds. They have significant concentrations of catechin and epicatechin-containing polyphenol proanthocyanidins, which are oligomers of flavan-3-ol molecules (Weseler & Bast, 2017).

Grape seeds have demonstrated potential as novel microbial agents mainly due to their abundance of polyphenols. Defatted grape seed extracts were demonstrated to have antibacterial action against *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Bacillus coagulans, Escherichia coli,* and *Pseudomonas aeruginosa*, according to Jayaprakasha et al (Jayaprakasha et al., 2003). A second study by Brown et al. found that muscadine grape seed extracts were efficient in suppressing *H. pylori* in vitro (Brown et al., 2009). Resveratrol exhibits antimicrobial activity by causing oxidative damage to bacterial membranes without harming host cells, particularly in *E. coli*. These findings provided insight into the potential of Resveratrol to support conventional treatments when antibiotics failed or proved inefficient (Subramanian et al., 2014) The polymeric phenolic fractions of Grape seed extract also showed the strongest specific inhibitory activity for practically all *Listeria* species; it has been reported (RHODES et al., 2006).

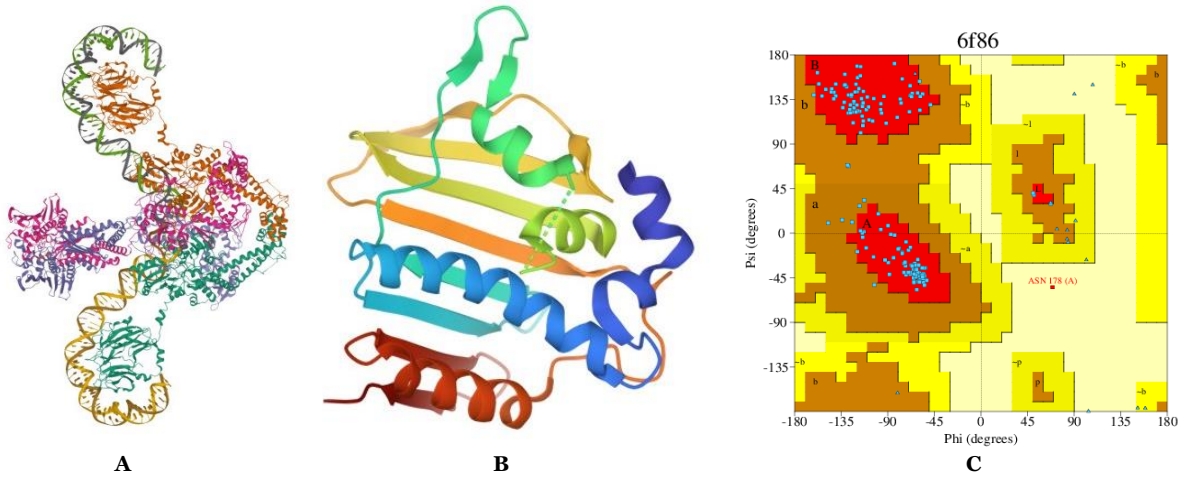


Figure 1. **A.** CryoEM structure of the complete E. coli DNA Gyrase complex bound to a 130 bp DNA duplex **PDB DOI:** *10.2210/pdb6RKW/pdb*_**B.** Crystal Structure of E. coli Gyrase subunit B **C.** Ramachandran plot of DNA Gyrase subunit B.

DNA gyrase is a type II topoisomerase that regulates the topological state of DNA in cells. It is made up of the subunits GyrA and GyrB. (Figure 01) (Wang, 2009). DNA supercoiling, which is necessary to maintain DNA topology during replication, is paired with ATP hydrolysis by the GyrB subunit via DNA gyrase. Across all bacterial species, it is a crucial enzyme whose inhibition causes a disturbance in DNA synthesis and, ultimately, cell death. Antibacterial medications have long been known to have an interest in the target DNA gyrase (Maxwell & Lawson, 2003). Quinolones and aminocoumarins are two families of antibiotics that have demonstrated in clinical studies that DNA gyrase is a workable target (Drlica et al., 2008).

MATERIALS AND METHODS

Data Collection and Ligand Preparation

A literature survey was done to select the suitable secondary metabolites. The 3D chemical structures of the selected secondary molecules were retrieved from PubChem database (PubChem (nih.gov)) in .sdf format. They were imported into Avogadro v.1.2.0 software and adjusted to physiological protonation states followed by energy minimization in the universal force field (conjugate gradient algorithm for 500 steps). Prepared ligands were saved in pdbqt format. OpenBableGUI is used for extension conversation.

Protein preparations

Crystal Structure of *E. coli* GyraseB (24kDa) – PDB ID-6F86 were obtained from protein data bank in .pdb format. Protein preparation was done using Autodocktools version 1.5.6. The water molecules and heteroatoms (CWW) were deleted. Missing atoms were repaired. Polar hydrogens and kollman charges were added to the protein.

Molecular Docking

The grid parameters and map files were created using Autogrid 4.2. to perform the blind Docking. (Genetic algorithm parameters settings: number of genetic algorithm (GA) runs: 50, population size: 300, the maximum number of evaluations: 25 000 000 (medium), and maximum number of generations: 27 000). Molecular Docking was performed using Autodock 4.2, and results were generated in .dlg format (output – Lamarckian GA-4.2).

Interaction Visualization and Analysis

Docking results were analyzed using AutoDockTools version 1.5.6 to examine the binding energies and Inhibition constants. Binding interactions with the ligand were visualized using UCSF Chimera 1.16 and PyMOL v.2.5.4. Protein-Ligand interaction profiler (PLIP - Welcome (tu-dresden.de)) and Proteins.plus (Zentrum für Bioinformatik: Universität Hamburg - Proteins Plus Server) web servers were also used to further analyze the binding residues.

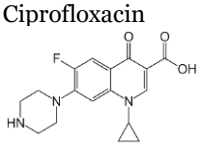
In silico Physicochemical and Pharmacokinetic Parameter Prediction

Veber's rules (Veber et al., 2002) and Lipinski's rule of five (Lipinski et al., 2001) were applied to evaluate drug-likeness using the swissADME server (SwissADME) . Secondary metabolites given in table 1 were analyzed for their physicochemical properties, and the results are epitomized in table 2. The ADMET characteristics of the Secondary metabolites were studied using the pkCSM server (https://biosig.lab.uq.edu.au/pkcsm/) to understand their pharmacokinetic Parameters, and the results are given in table 3.

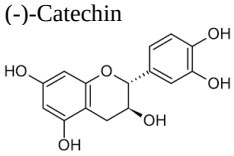
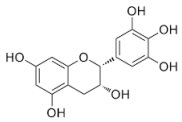
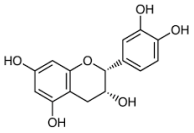
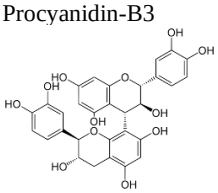
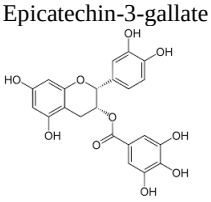
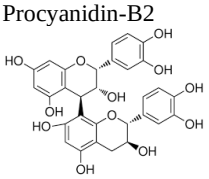
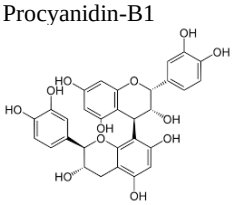
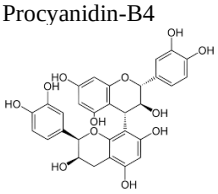
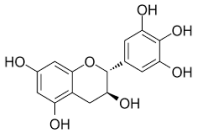
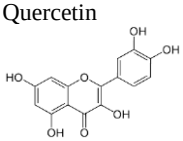
RESULTS AND DISCUSSION

Table 1. Calculated binding energies and inhibition constants of a. ciprofloxacin-a and b. selected phytochemicals-b.

a.

Reference Compound	Binding Energy / kcal/mol	Ki-Inhibition constant/ µM
Ciprofloxacin 	-6.19	28.80

b.

Compound	Binding Energy / kcal/mol	Ki-Inhibition constant /µM	Compound	Binding Energy / kcal/mol	Ki-Inhibition constant /µM
(-)-Catechin 	-6.22	27.52	Epigallocatechin 	-5.11	180.46
(-)-Epicatechin 	-5.32	95.83	Procyanidin-B3 	-5.59	80.03
Epicatechin-3-gallate 	-4.46	540.36	Procyanidin-B2 	-6.31	23.67
Procyanidin-B1 	-5.05	198.11	Procyanidin-B4 	-5.60	77.90
(+)-Gallocatechin 	-5.31	127.21	Quercetin 	-5.36	117.82

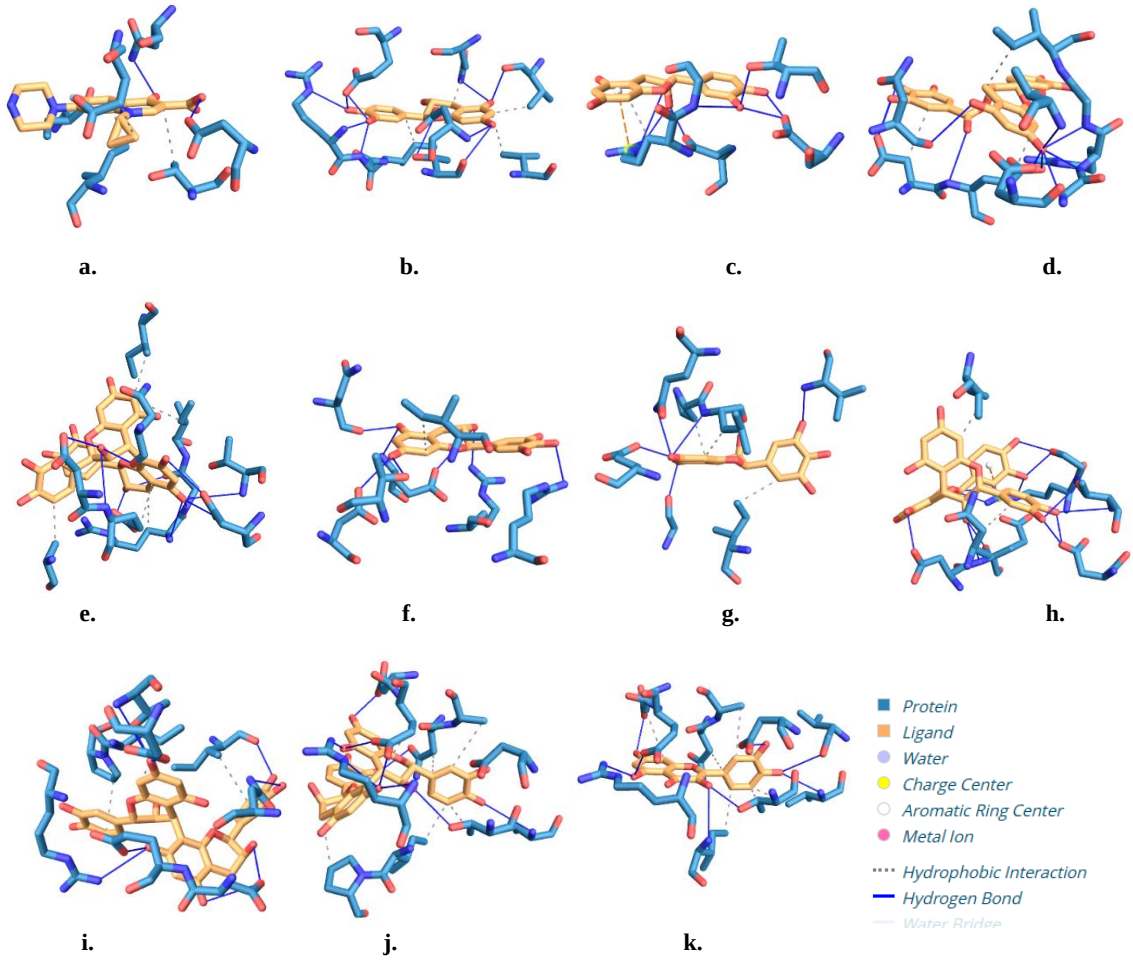


Figure 2. Bonding interaction within the binding pocket of DNA GyrB with **a.** Ciprofloxacin **b.** (-)-catechin **c.** Epicatechin **d.** Epicatechin **e.** Procyanidin-B1 **f.** (+)-Gallocatechin **g.** Epigallocatechin **h.** Procyanidin-B3 **i.** Procyanidin-B2 **j.** Procyanidin-B4 **k.** Quercetin that generated using Protein-Ligand interaction profiler.

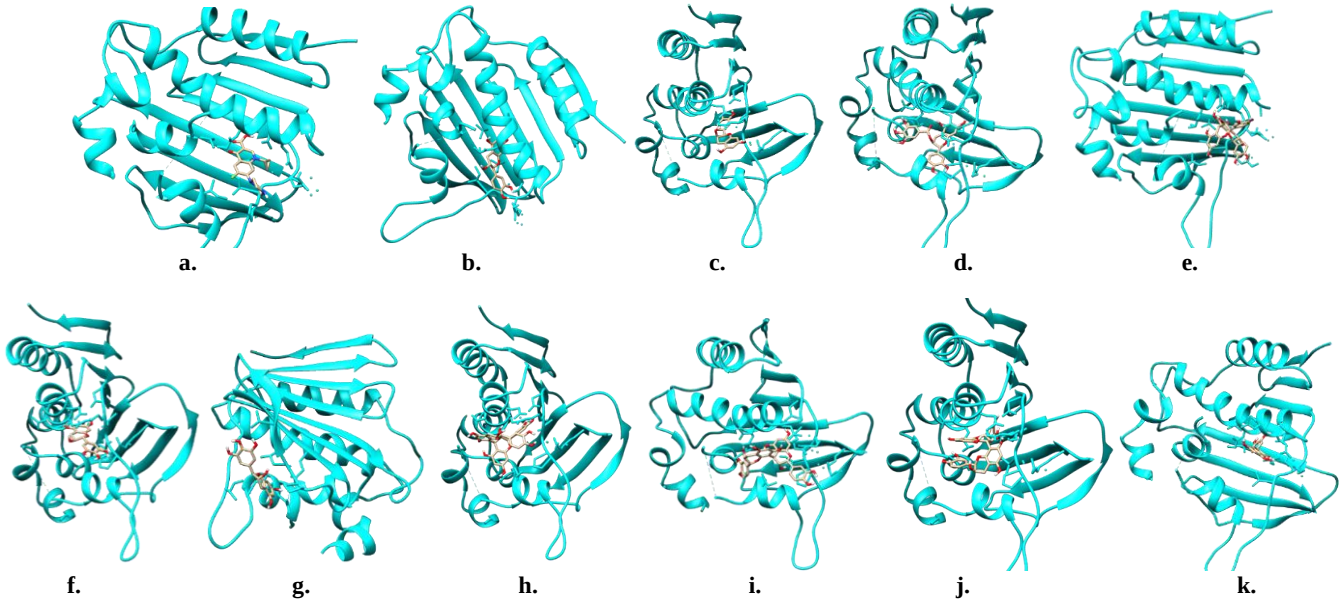
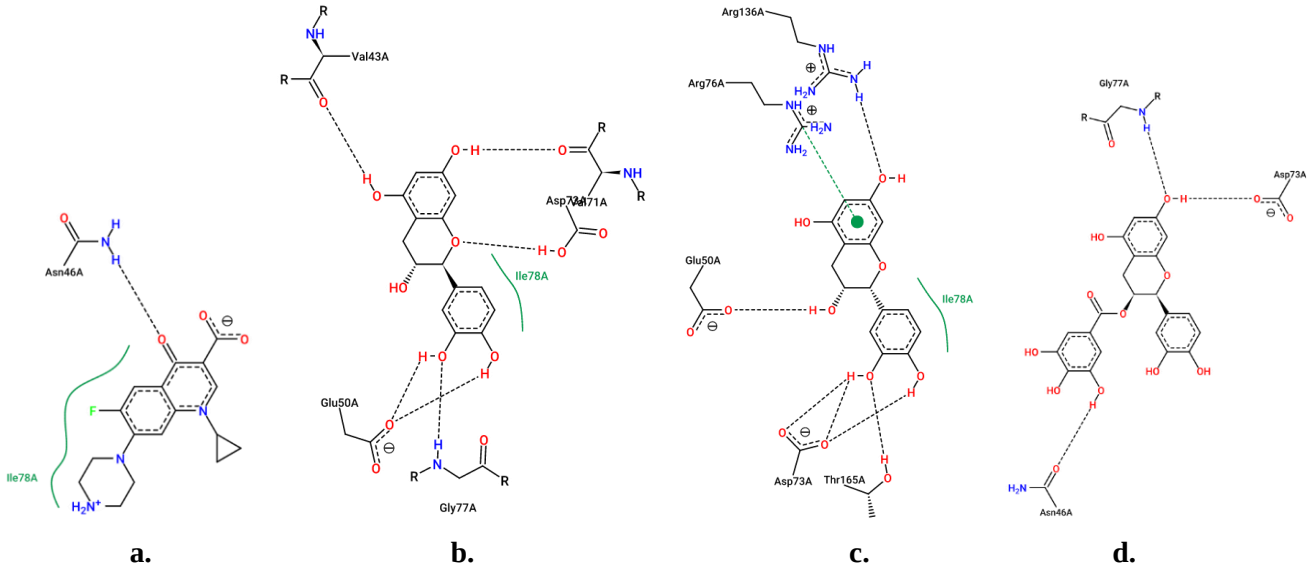
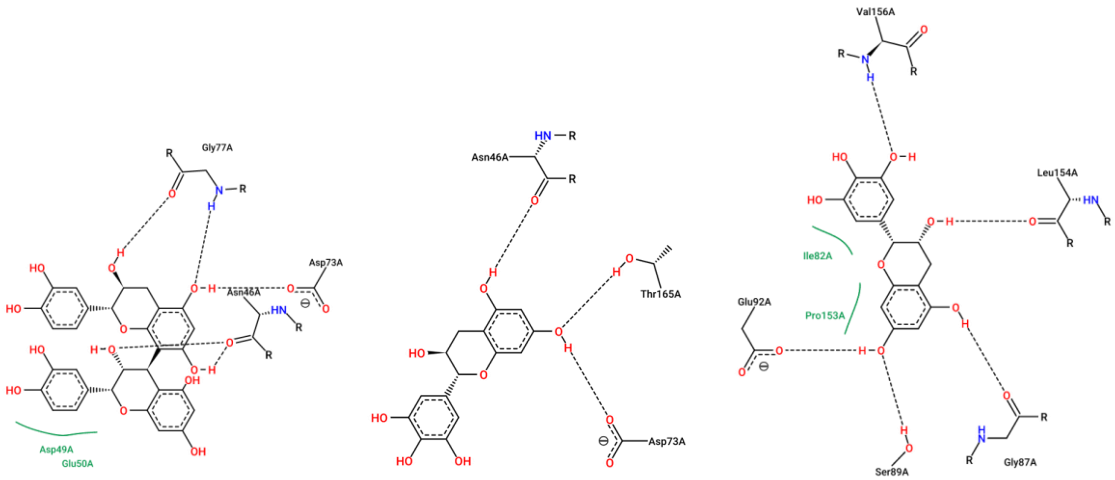


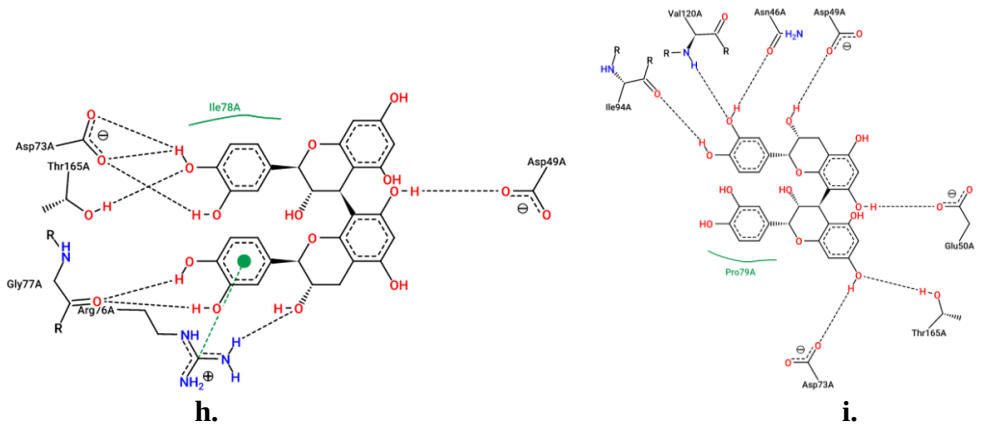
Figure 3. 3D visualization of the ligand-DNA GyrB complexes, **a.** Ciprofloxacin **b.**(-)-catechin **c.** Epicatechin **d.** Epicatechin **e.** Procyanidin-B1 **f.** (+)-Gallocatechin **g.** Epigallocatechin **h.** Procyanidin-B3 **i.** Procyanidin-B2 **j.** Procyanidin-B4 **k.** Quercetin obtained from Protein-Ligand interaction profiler.


Asn46A
Ile78A
Val43A
Asp73A
Val71A
Glu50A
Gly77A
Arg136A
Arg76A
Asp73A
Thr165A
Gly77A
Asn46A

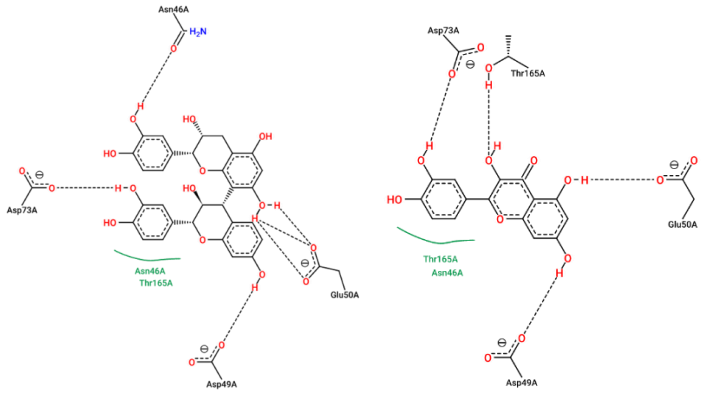
a. **b.** **c.** **d.**


Gly77A
Asp73A
Asn46A
Asp49A
Glu50A
Thr165A
Val156A
Leu154A
Ile82A
Glu92A
Pro153A
Ser89A
Gly87A

e. **f.** **g.**


Ile78A
Asp73A
Thr165A
Asp49A
Gly77A
Arg76A
Val120A
Asn46A
Asp49A
Ile94A
Glu50A
Pro79A
Thr165A
Asp73A

h. **i.**



j. **k.**

Figure 4. Diagram showing the amino acid residues that interact with ligand, **a.** Ciprofloxacin **b.** (-)-catechin **c.** Epicatechin **d.** Epicatechin **e.** Procyanidin-B1 **f.** (+)-Gallocatechin **g.** Epigallocatechin **h.** Procyanidin-B3 **i.** Procyanidin-B2 **j.** Procyanidin-B4 **k.** Quercetin that generated using Protein-Ligand interaction profiler.

Table 2. Predicted pharmacokinetic properties of studied secondary metabolites; 1-Water solubility (log mol/L) 2-Human Intestinal absorption (% Absorbed) 3-volume of distribution at steady-state (log L/kg) 4-Fraction unbound (Fu) 5-Blood brain barrier permeability (log BB) 6-Cytochromes P450 substrates 7- Cytochromes P450 inhibitors 8- Total Clearance (log ml/min/kg) 9- Renal Organic cation transporter 2 substrate 10- AMES toxicity 11- Max. tolerated dose (human) (log mg/kg/day) 12- Hepatotoxicity.

Compound	Absorption		Distribution			Metabolism		Excretion		Toxicity		
	WS_1	HIA_2	$VDss_3$	FU_4	BBB_5	$CYPS_6$	$CYPI_7$	TC_8	ROS_9	$AMES_{10}$	MTD_{11}	HT_{12}
Ciprofloxacin	-2.678	94.828	0.293	0.404	-0.733	3A4	-	0.505	No	Yes	-0.283	Yes
(-)-Catechin	-3.024	72.536	0.589	0.152	-1.278	-	-	0.286	No	Yes	0.516	No
(-)-Epicatechin	-3.024	72.539	0.589	0.152	-1.278	-	-	0.286	No	Yes	0.516	No
Epicatechin-3-gallate	-2.907	66.671	0.051	0.283	-1.898	-	1A2	0.016	No	Yes	0.537	No
Procyanidin-B1	-2.892	64.165	0.036	0.386	-2.263	-	-	0.289	No	No	0.411	No
(+)-Gallocatechin	-2.952	60.140	0.520	0.156	-1.598	-	-	0.449	No	No	0.755	No
Epigallocatechin	-2.952	60.140	0.520	0.156	-1.598	-	-	0.449	No	No	0.755	No
Procyanidin-B3	-2.892	64.165	0.036	0.386	-2.263	-	-	0.289	No	No	0.411	No
Procyanidin-B2	-2.892	64.165	0.036	0.386	-2.263	-	-	0.289	No	No	0.411	No
Procyanidin-B4	-2.892	64.165	0.036	0.386	-2.263	-	-	0.289	No	No	0.411	No
Quercetin	-2.982	74.840	0.310	0.128	-1.377	-	1A2	0.624	No	Yes	0.954	No

Table 3. Predicted Physicochemical properties of studied secondary meabolites ; 1- Molecular weight (g/mol) 2- Num. heavy atoms 3- Num. rotatable bonds 4- Num. H-bond acceptors 5- Num. H-bond donors 6-Topological polar surface area(Å²) 7- Octanol/water partition-coefficient 8-Lipinski's rule of five 9- Veber's rule.

Compound	MW^1	$n\text{-}nha^2$	$n\text{-}rot^3$	$n\text{-}Hba^4$	$n\text{-}Hbd^5$	$TPSA^6$	$MiLogp^7$	Lpi^8	Ver^9	BAS^{10}
Ciprofloxacin	331.34	24	3	5	2	74.57	1.28	Yes	Yes	0.55
(-)-Catechin	290.27	21	1	6	5	110.38	0.24	Yes	Yes	0.55
(-)-Epicatechin	290.27	21	1	6	5	110.38	0.24	Yes	Yes	0.55
Epicatechin-3-gallate	442.37	32	4	10	7	177.14	0.32	Yes	No	0.55
Procyanidin-B1	578.52	42	3	12	10	220.76	-0.26	No	No	0.17
(+)-Gallocatechin	306.27	22	1	7	6	130.61	-0.29	Yes	Yes	0.55
Epigallocatechin	306.27	22	1	7	6	130.61	-0.29	Yes	Yes	0.55
Procyanidin-B3	578.52	42	3	12	10	220.76	-0.26	No	No	0.17
Procyanidin-B2	578.52	42	3	12	10	220.76	-0.26	No	No	0.17
Procyanidin-B4	578.52	42	3	12	10	220.76	-0.26	No	No	0.17
Quercetin	302.24	22	1	7	5	131.36	-0.56	Yes	Yes	0.55

DISCUSSION

In this study, ciprofloxacin was used as the reference compound, the known bacterial DNA gyrase subunit B inhibitor, to compare the binding energies and inhibition constants with the examined secondary metabolites. The concentration needed to cause half of the maximum inhibition is known as the inhibition constant (Ki), which serves as a measure of an inhibitor's potency. When comparing the binding energies of docked ligands and reference drug, (-)-catechin (-6.22 kcal/mol) and Procyanidin-B2(-6.31 kcal/mol) exhibit even better binding affinity to the target protein than ciprofloxacin (-6.19 kcal/mol). (Table 01) Also, those two compounds show lower Ki values than ciprofloxacin, indicating better potency towards the GyrB inhibition. It was evident that the top-ranked phytochemical structures' hydroxyl groups play a substantial role in the establishment of potent hydrogen bonds with amino acid residues in the binding pockets. (Figure 02)

When considering the binding interactions of those three ligands, ciprofloxacin shows only one Hydrogen bonding interaction with the protein (with Asn46). But (-)-catechin and Procyanidin-B2 shows six (with Val43, Asp72, Gly77 & Glu50) and seven (with Ile94, Val120, Asn46, Asp49, Glu50, Thr165 & Asp73) (Figure 3) hydrogen bonding interactions with the target protein, respectively. Epicatechin-3-gallate shows the lowest (-4.46 kcal/mol) binding affinity towards the target protein and the highest inhibition constant (540.36 µM) from the examined ligands. Although it is not mush as (-)-catechin and Procyanidin-B2, phytochemicals Procyanidin-B3(-5.59 kcal/mol), B4(-5.60 kcal/mol), (-)-Epicatechin (-5.32 kcal/mol) and Quercetin (-5.36 kcal/mol) also exhibits good binding capabilities. Major types of binding interactions between the protein and the ligands are H-bonding and Hydrophobic interactions. Other than that, π-Cation Interactions also can be observed in two cases which are (-)-Epicatechin and Procyanidin-B3. Interestingly in both cases, π-Cation Interactions have occurred between the aromatic rings in the ligands and the Arg76 amino acid.

From the leading phytochemicals observed with the highest binding affinities, (-)-Catechin meets Lipinski's and Veber's rules, fulfilling strong physicochemical requirements (Table 3) as a potential drug. Also (-)-Catechin shows a satisfactory percentage of absorption through the human intestinal tract. Another important characteristic is that all the screened phytochemicals from grape seed extract don't show hepatotoxicity (Table 2), even though the well-known commercially available antibiotic ciprofloxacin shows some level of hepatotoxicity. In pharmacokinetics and toxicity analysis, it is categorized that values between -4 and -2 log mol/L as water "soluble "compounds. Interestingly all the compounds examined are coming under that category. Out of the phytochemical from the grape seed extract assessed, no chemical acted as a substrate for Cytochromes P450 isozymes but the reference antibiotic. However, predicted data revealed that Epicatechin-3-gallate and Quercetin have the potential to inhibit CYP1A2 isozyme.

CONCLUSIONS

Selected secondary metabolites found in Grape seed extract were tested *in silico* for their potential inhibitory action towards DNA Gyrase enzyme, pharmacokinetic and Physicochemical properties, and compared with the commercial antibiotic, ciprofloxacin. Out of the ten-phytochemical assessed, (-)-catechin and Procyanidin-B2 indicated the best receptor inhibition capabilities. Comprehensive docking, pharmacokinetic, and physicochemical tests indicated that (-)-Catechin would be a viable DNA gyrase B inhibitor because it binds firmly to the binding pocket, inhibiting the native conformation of the protein. Hence, (-)-Catechin is an exceptional candidate for further *in vitro/in vivo* studies.

***Authors' Contributions*:** P.L.M.J.H. Lawan and D.H. Tharakee designed the study, carried out computational studies, and analyzed the data. P.L.M.J.H. Lawan and D.H. Tharakee wrote the manuscript. All authors read and approved the final version of the manuscript.

***Competing Interests*:** The authors declare that there are no competing interests.

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