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In Silico Analysis of *Moringa oleifera* Leaf Phytochemicals as Potential DNA Gyrase Inhibitor in *Salmonella typhi*

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ABSTRACT

Salmonella typhi is an infectious bacterium leading to typhoid fever, that is increasing in cases worldwide each year. Due to the resistance to antibiotics, the discovery of safer and efficacious drugs remains important. This study evaluates the potential interaction of M. oleifera phytochemical compounds against DNA gyrase of Salmonella typhi using an in silico molecular docking approach. Ligand's phytochemicals were retrieved from the PubChem database, while the three-dimensional structure of DNA Gyrase subunit A and subunit B were obtained from the Protein Data Bank (PDB ID: 5ZJT and 6J90). Molecular docking was done using PyRx software and BIOVIA Discovery Studio as visualization software. Phytochemical compounds that had higher predictive binding affinity to DNA gyrase subunit A were Naringenin (ΔG=-7.9 kcal/mol), 6-Prenylnaringenin (ΔG=-8.1 kcal/mol), 6-Methoxypodophyllotoxin (ΔG=-7.9 kcal/mol). Brefeldin A-DNA gyrase subunit A complex had the same binding affinity to ciprofloxacin (ΔG=-7.7 kcal/mol). Phytochemical compounds that had higher predictive binding affinity to DNA gyrase subunit B were Naringenin (ΔG=-8.3 kcal/mol), 6-Prenylnaringenin (ΔG=-9.4 kcal/mol), 3',4'-Dimethoxy-7-hydroxyflavone (ΔG=-8.5 kcal/mol), Sinapoyl malate (ΔG=-8.3 kcal/mol), and Malvidin ((ΔG=-8.8 kcal/mol). These findings suggest that phytochemical compounds from M. oleifera leaves may act as potential DNA gyrase inhibitors. However, further in vitro and in vivo validation is required to confirm its antibacterial activity.

1. INTRODUCTION

Typhoid fever is a severe systemic disease in humans, reservoir by Salmonella typhi, the causative agent. *S. typhi* produces typhoid toxin indicated primary virulence factor that caused typhoid fever (Ecdc, n.d.; Fowler & Galán, 2018). Many symptoms caused are headache, nausea, vomiting, constipation, sometimes diarrhea and anorexia (Ahmad et al., 2026). Typhoid fever occurs globally with a number of around 11-21 million cases each year. Among those numbers, it is estimated 130.000-160.000 related to deaths (Ecdc, n.d.). This might be caused by the resilience of *S. typhi* in the environment, such as water and food ranging from 14 to 140 days. The bacterium can also form biofilm, a nutrient-rich environment, to survive and protect itself (Buzilă et al., 2025).

S. typhi is considered one of the most prevalent isolated food-borne infections (Gaikwad et al., n.d.). It is because the major transmission is contaminated food or water from the urine or feces of infected patients or asymptomatic carriers (Buzilă et al., 2025). In 1970s, first line antibiotics treatment (ampicillin, chloramphenicol and cotrimoxazole) is still effective, but those are ineffective to typhoid fever treatment recently (Asghar et al., 2024). The use of antibiotics faces challenges and difficulties due to the resistance of microorganisms. The emergence of *S. typhi* Multi Drug Resistant (MDR) is ineffective to fluoroquinolone due to the genetic mutation. Moreover, the emergence of extensively drug-resistant (XDR) of *S. typhi* also makes the resistance to fluoroquinolone (Ahmad et al., 2026). In addition, the side effects of prolonged antibiotic therapy increase the higher risk to the human body(Gaikwad et al., n.d.). Therefore, the use of

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plants as alternative medicine, safer and cheaper, is a good decision as a candidate for future medicine with low risks.

In ancient times, plants were the main medicine for natural remedies. As human knowledge increases, plants are used as a major source of herbal medicine around 70-80% of the world's population (Gaikwad et al., n.d.). The plants have been widely used to treat wound infections, malaria, diabetes, anemia, and other conditions. More than 90 compounds have been identified in Moringa genus based on literature studies from 2010 to 2022 (Pareek et al., 2023). Several studies claim that the plants contain various phytochemical compounds like tannin, flavonoid, phenolic compounds, and alkaloids, which have a role in pharmacological activities, including antimicrobial agents (Akinseye et al., 2025).

Moringa oleifera, a tropical and sub-tropical plant, is commonly known as a "super food" due to its various nutrients and phytochemical compounds. This plant is abundant in many countries because of its ability to survive in dry environment. Plant parts of *M. oleifera* reveal different pharmacological activities such as anti-inflammatory, antioxidant, cardioprotective (seed), antioxidant, immunomodulatory, cardiovascular (stem) and treatment for urinary tract infections (flower) (Pareek et al., 2023). *M. oleifera* leaves contain many phytochemical compounds higher and more varied than other parts. Leaves extract of *M. oleifera* exhibit antioxidant, antibacterial, antimicrobial and anti-inflammatory properties (Chakraborty et al., 2025; Osorio et al., 2021; Pareek et al., 2023).

Based on the previous study, *M. oleifera* leaves extract exhibit antibacterial activity against Salmonella typhi in a rat model (Nkamkeu et al., 2025). Methanolic extract of *M. oleifera* leaves had high antibacterial activity with 15±0.42 inhibition zone of *S. typhi* (El-Sherbiny et al., 2024). Another study revealed that *M. oleifera* also has antibacterial potential against Escherichia coli using molecular docking approach targeting DNA gyrase B, Dihydropteroate synthase, Enoyl acyl carrier protein reductase, FIMH, Par E topoisomerase IV (Akinseye et al., 2025). Consumption of *M. oleifera* extract is safe at the doses because the researchers widely used in developing various formulation (Pareek et al., 2023).

DNA gyrase of *S. typhi* is a prominent enzyme that supports negative supercoiling of DNA, facilitating the transcription and replication process (Ameji et al., 2025). Previous study exhibited ciprofloxacin analogs potent as DNA gyrase inhibitors (Hasan et al., 2021). Retardation of this enzyme makes an unstable DNA-enzyme complex form and leads to cell death due to DNA poisoning (Ameji et al., 2025). DNA gyrase subunit B plays crucial role in bacterial replication. Inhibition of this subunit makes replication interrupted (Akinseye et al., 2025). DNA gyrase subunit A is liable in binding and wrapping DNA (Rajakumari et al., 2024). Previous study between *M. oleifera* and DNA gyrase of *E. coli* had been done. But, research on *M. oleifera* targeting *S. typhi* DNA gyrase using molecular docking remains limited. Consequently, the purpose of this study is to evaluate the potential *M. oleifera* phytochemical compounds against DNA gyrase of *S. typhi* using molecular docking approach.

2. METHODS

This study used a molecular docking approach to assess the binding of phytochemical compounds of Moringa oleifera leaves as ligands to the DNA gyrase of *S. typhi* as the receptor. The inclusion criteria involve phytochemical compounds of *M. oleifera* methanolic extract that fulfilled Lipinski's Rule of Five, DNA gyrase subunit A and B as receptor (target protein), and ciprofloxacin as reference compound (Chakraborty et al., 2025; Sharma et al., 2017). Three-dimensional structures of DNA gyrase were downloaded from the RCSB Protein Data Bank (RCSB PDB: https://www.rcsb.org/), under the codes 5ZTJ (DNA gyrase subunit A) and 6J90 (DNA gyrase subunit B). The cleaning process of the target protein structure from water molecules and heteroatoms was done using PyMOL software and saved to .pdb format.

A number of *M. oleifera* leaves compounds as the ligand were obtained from LC-MS-ESI profiling from the previous study (Chakraborty et al., 2025). Ciprofloxacin is used as a positive control due to the ability to bind DNA gyrase (Hasan et al., 2021). Ligand (67 compounds) and ciprofloxacin structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

Selected ligands were evaluated by Lipinski's Rules of Five using SWISS ADME software (https://www.swissadme.ch/). Molecular docking between DNA gyrase and *M. oleifera* phytochemical compounds has been performed using the PyRx v8.0 on Autodock Vina tools (Exhaustiveness = 8) (Dallakyan and Olson, 2015). Setting the center of the grid box to; x=26.3791 Å, y=22.9034 Å, z=22.0193 Å and dimension to; x=51.5378 Å, y=53.7244 Å, z=51.0905 Å was used to evaluate the binding ligand and DNA gyrase subunit A. In addition, setting the center of the grid box to; x=-11.903 Å, y=35.6167 Å, z=28.2708 Å and dimension to; x=54.4239 Å, y=77.3522 Å, z=59.3428 Å was used to evaluate the binding ligand and DNA gyrase subunit B. This process is used to identify the minimum energy conformations of the binding poses of ligand. There were 9 binding poses resulting from the molecular docking process. The best pose was chosen based on RMSD upper or lower bond value of 0.0.

Re-docking was performed to validate the parameters used for docking the native ligand with the receptor. The native ligand was bound to the receptor and separated before the redocking process. The overlay method of the docking result structure with the native crystal structure aims to validate the accuracy of the docking method by calculating The Root Mean Square Deviation (RMSD. Re-docking was done using BIOVIA Discovery Studio (File version 25.1.0.0), observing RMSD value, with a standard value of <2.00 Å. These parameters were used for knowing the stability of the structure during molecular docking process (Agu et al., 2023a; Terefe & Ghosh, 2022).

The minimum-energy conformations were identified as those with the highest binding affinity score. Inhibitor candidate compounds that have the potential to bind DNA gyrase are determined based on ligands with the binding affinity scores exceeding ciprofloxacin as a positive control. BIOVIA Discovery Studio (File version 25.1.0.0) was used as a visualization of molecular docking's results to evaluate the amino acid residues (Agu et al., 2023a). Analysis was also carried out based on the bonds formed and amino acid residues by comparison with amino acids bound to ciprofloxacin and the native ligand of DNA gyrase. SWISS Target Prediction (https://www.swisstargetprediction.ch/) was used as target confirmation of the ligand's binding to the target protein. Ligands that have the potential to be DNA gyrase inhibitor candidates are ranked based on the binding affinity score that is higher than ciprofloxacin as a positive control.

3. RESULT AND DISCUSSION

Result

In the previous study, a total bioactive compound of *Moringa oleifera* methanolic leaves extract were 67 compounds from various groups of phenolic acids, flavonoids, glycosides, and alkaloid (Chakraborty et al., 2025). A total of 13 phytochemical compounds fulfilled Lipinski's Rules of Five (Table 1). The structural data of ligands derived from PubChem and Swiss ADME online software. Removing the water molecules and heteroatoms was done using PyMOL software.

Table 1. List of Phytochemical Compounds in *M. oleifera* Methanolic Leaves Extract

No	Phytochemicals	PubChem ID	Log P <5	H donor (<5)	H acceptor (<10)	Molar refractivity (40-130)	Molecular weight (<500 g/mol)
1	Naringenin	439246	1.84	3	5	71.57	272.25
2	Demethyl medicarpin	3347979	2.14	2	4	68.70	265.25
3	Malvidin	159287	0.92	4	7	87.30	330.30
4	3 ', 4 ' - D i m e t h o x y - 7 - Hydroxyflavone	5378518	2.79	1	5	82.93	298.29
5	5-Methoxysalicylic Acid	75787	1.14	2	4	41.92	168.15
6	6-Prenylnaringenin	155094	3.27	3	5	95.29	340.4
7	Sinapoyl malate	14605050	1.07	2	8	78.83	340.28
8	Scopoletin	5280460	1.52	1	4	51.00	192.17
9	Sinapoyl malate-4'-methyl Ester	134780544	1.27	2	9	84.72	354.31
10	Scopolamine	638340	1.57	1	5	83.48	303.35

11	6-Methoxypodophyllotoxin	3035544	2.34	1	9	110.34	444.43
12	Bilobalide	73581	0.16	2	8	71.20	326.30
13	Brefeldin A	5287620	1.86	2	4	77.46	280.36

Molecular docking between DNA gyrase and *M. oleifera* phytochemical compounds to identify the minimum energy conformations of the binding poses of ligand. The binding energy of phytochemical compounds of *M. oleifera* leaves and the target protein is presented on Table 2. Redocking the native ligand with the receptor indicated the stability of the structure during molecular docking process. Native ligand for DNA gyrase subunit A (PDB ID: 5ZTJ) was O54 and for DNA gyrase subunit B was ATP. RMSD value showed <2.00 Å for both. The binding affinity of the native ligand and DNA gyrase subunit A is ΔG = -8.0 kcal/mol, while subunit B is ΔG = -7.5 kcal/mol.

Table 2. List of The Binding Energy of *M. oleifera* Phytochemicals to DNA gyrase

No		**Binding Affinity**	
		DNA Gyrase subunit A	**DNA Gyrase subunit B**
1	Ciprofloxacin	-7.7	-7.5
2	Naringenin	-7.9	-8.3
3	Demethyl medicarpin	-7.6	-6.8
4	Malvidin	-7.5	-8.8
5	3',4'-Dimethoxy-7-hydroxyflavone	-7.5	-8.5
6	5-Methoxysalicylic Acid	-5.4	-6.7
7	6-Prenylnaringenin	-8.1	-9.4
8	Sinapoyl malate	-6.5	-8.3
9	Scopoletin	-5.9	-6.9
10	Sinapoyl malate-4'-methyl ester	-6.4	-6.6
11	Scopolamine	-6.9	-6.8
12	6-Methoxypodophyllotoxin	-7.9	-6.7
13	Bilobalide	-7.6	-6.5
14	Brefeldin A	-7.7	-7.2

Ciprofloxacin exhibited the interactions to DNA gyrase subunit A (ΔG = -7.7 kcal/mol) and DNA gyrase subunit B (ΔG = -7.5 kcal/mol). Phytochemical compounds that had higher binding affinity to DNA gyrase subunit A than ciprofloxacin were Naringenin (ΔG = -7.9 kcal/mol), 6-Prenylnaringenin (ΔG = -8.1 kcal/mol), and 6-Methoxypodophyllotoxin (ΔG = -7.9 kcal/mol). Only 6-Prenylnaringenin (ΔG = -8.1 kcal/mol) that higher binding affinity than native ligand to DNA gyrase subunit A. Phytochemical compounds that had higher binding affinity to DNA gyrase subunit B were Naringenin (ΔG = -8.3 kcal/mol), 6-Prenylnaringenin (ΔG = -9.4 kcal/mol), 3',4'-Dimethoxy-7-hydroxyflavone (ΔG = -8.5 kcal/mol), Sinapoyl malate (ΔG = -8.3 kcal/mol), and Malvidin (ΔG = -8.8 kcal/mol).

The docking process visualization in this study used Discovery Studio software developed by Dassault Systemes BIOVIA. This software not only visualizes docking results but also analyzes them and combines the binding modes of different ligands to the same target (Agu et al., 2023b). The molecular docking visualization results are shown in Figures 1 and 2 below.

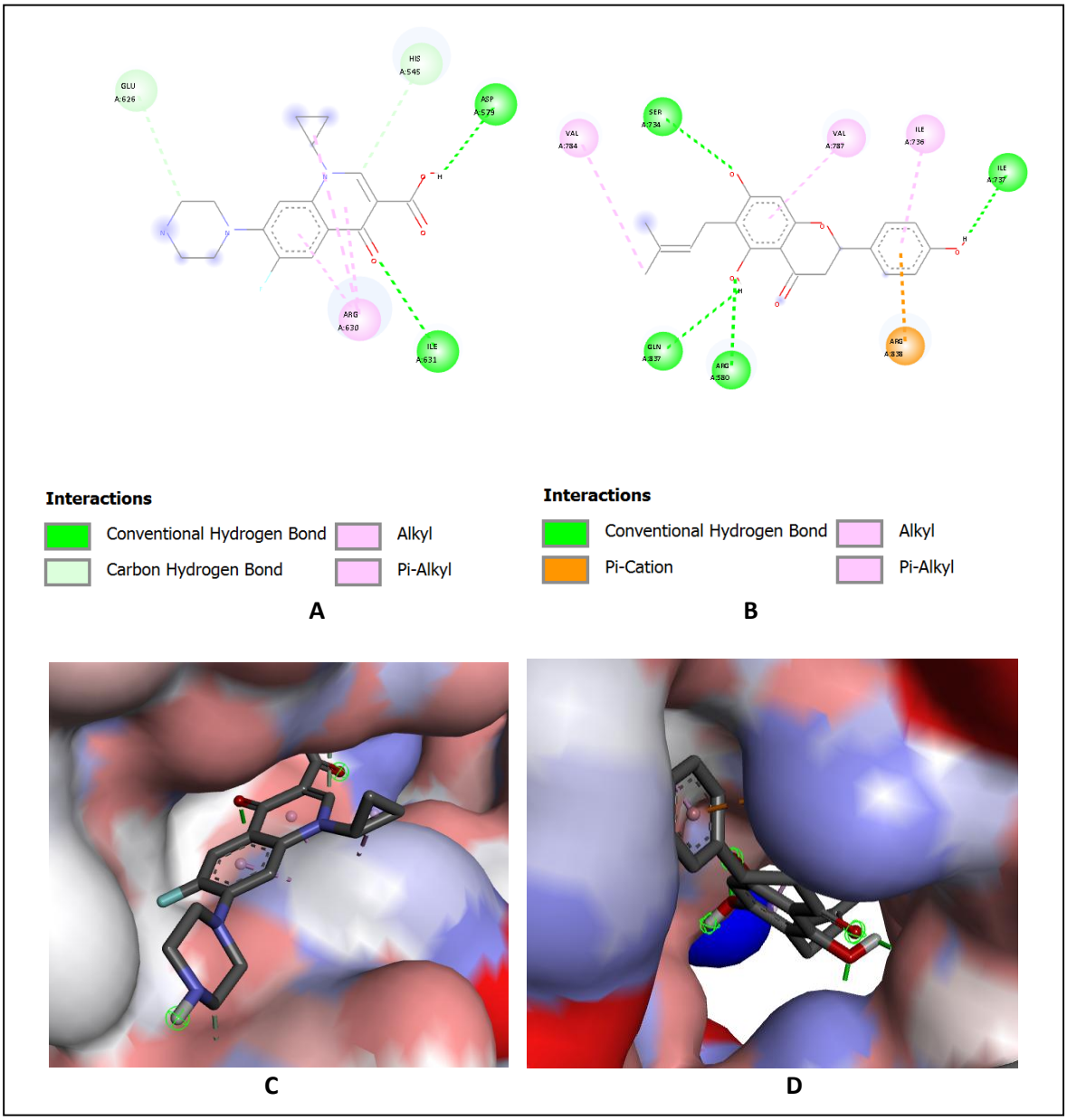

Figure 1. Visualization of ligand and subunit A of DNA gyrase. (A) 2D diagram of Ciprofloxacin-DNA gyrase subunit A complex (B) 2D diagram of 6-Prenylnaringenin-DNA gyrase subunit A complex (C) Binding site Ciprofloxacin-DNA gyrase subunit A complex (D) Binding site 6-Prenylnaringenin-DNA gyrase subunit A complex.

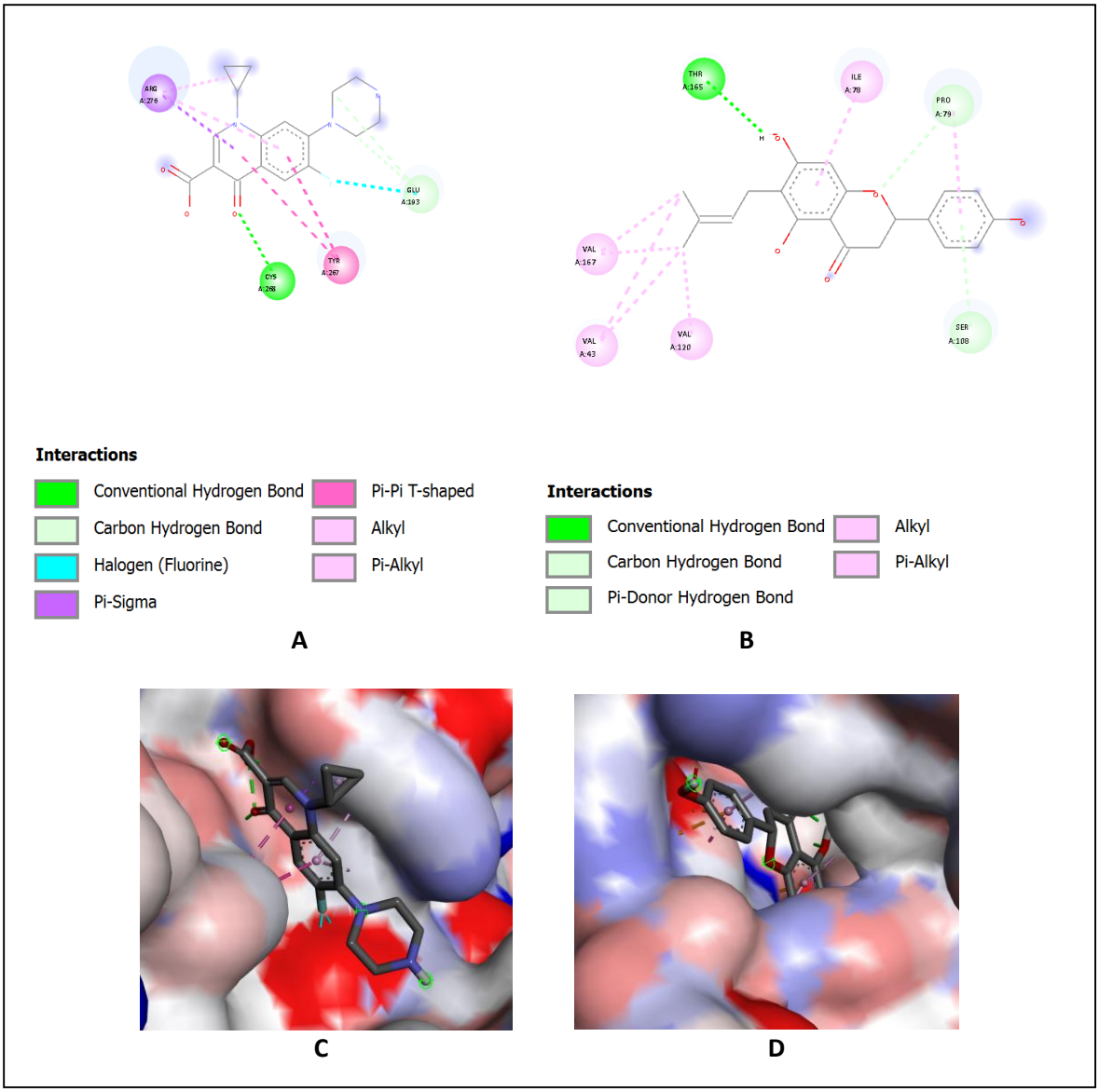

Figure 2. 2D diagram of the interaction ligand and subunit B of DNA gyrase. (A) Ciprofloxacin-DNA gyrase subunit B complex (B) 6-Prenylnaringenin-DNA gyrase subunit B complex (C) Binding site Ciprofloxacin-DNA gyrase subunit B complex (D) Binding site 6-Prenylnaringenin-DNA gyrase subunit B complex

The ability of a ligand to bind to a target protein can be determined by comparing the binding results with the native ligand and with a positive control. This is because the similarity of the binding sites indicates a high potential for a ligand to bind to the target protein. The results of ligand binding from *M. oleifera* phytochemical compounds to DNA gyrase are presented in Table 3 below.

Table 3. List of Amino Acid Residues

No	Phytochemicals	Amino Acid Residues	
		DNA Gyrase subunit A	DNA Gyrase subunit B
1	Native ligand	ARG 580; GLN 837; LEU 836; VAL 787; ASP 686; ARG 739; ILE 736; VAL 787	VAL 118; ALA 100; ASN 46; ASP 73; ALA 47; GLY 119; GLY 117; HIS 116; LYS 337; LEU 115; VAL 120; LYS 103; GLY 102; TYR 109; ILE 78; GLU 50
2	Ciprofloxacin	GLU 626; HIS 545; ASP 579; ARG 630; ILE 631	ARG 276; GLU 193; TYR 267; CYS 268

3	Naringenin	GLN 788; ARG 838; ILE 736; ARG 580; THR 632; VAL 685	ASP 73; ALA 47; ASN 46; ILE 78; TYR 109; ARG 76; PRO 79
4	Demethyl medicarpin	ILE 736; ARG 838; LEU 735; ARG 580; LEU 735; SER 734; ILE 683	GLU 363; LYS 223; ILE 240; ASN 364
5	Malvidin	VAL 685; ARG 580; SER 734; ALA 786; VAL 733; ASP 579; LEU 836; GLY 835	LYS 105; ASN 46; GLU 50; ASP 73; ILE 78; TYR 109; ILE 94; SER 121; VAL 120; GLY 119
6	3',4'-Dimethoxy-7-hydroxyflavone	VAL 620; ILE 631; GLU 626; ASN 628; ARG 630; ASP 579; HIS 545	ASN 46; PRO 79; ILE 78; TYR 109
7	5-Methoxysalicylic Acid	VAL 685; ILE 736; ILE 737; ARG 739; ASP 686; ARG 838; GLN 788	ILE 94; ALA 100; GLY 117; SER 121; VAL 120
8	6-Prenylnaringenin	VAL 784; SER 734; VAL 787; ILE 736; ILE 737; ARG 838; ARG 580; GLN 837	THR 165; ILE 78; PRO 79; VAL 167; VAL 43; VAL 120; SER 108
9	Sinapoyl malate	ARG 580; LEU 836; ALA 786; SER 734; ALA 633; ASP 636	THR 165; PRO 79; ALA 100; ALA 47; ILE 78; ASN 46; GLY 101; VAL 118; GLY 119; LYS 103; SER 121; VAL 120
10	Scopoletin	LEU 735; ASP 686; VAL 685; ARG 580	ILE 94; LYS 103; ASN 46; GLY 119; GLY 102; GLY 101
11	Sinapoyl malate-4'-methyl ester	ARG 580; LEU 836; LEU 735; VAL 787; ILE 736; GLN 788; ARG 833; SER 734	LYS 103; SER 108; ARG 136; ILE 94
12	Scopolamine	ILE 736; ASP 686; VAL 733; ARG 580	PRO 274; ARG 276; TYR 267; GLU 264; ILE 266
13	6-Methoxypodophyllotoxin	ARG 580; SER 734; ASP 686; ILE 736; ARG 739; ILE 737; ARG 838	GLN 335; HIS 38; THR 336; GLY 24; ARG 276; ASP 338; THR 34
14	Bilobalide	LEU 736; THR 632; ARG 580	GLY 35; ASP 29; THR 34
15	Befeldin A	VAL 733; ALA 786; SER 734; LEU 635	CYS 268; ARG 276; PRO 274

Discussion

Naringenin showed high predictive potential to bind to the DNA gyrase subunit A (ΔG = -7.9 kcal/mol) and subunit B (ΔG = -8.3 kcal/mol). Naringenin belongs to the flavonoid family, especially the flavanone (Veiko et al., 2023). Other compound, 6-Prenylnaringenin, the highest binding affinity, interacts with subunit A and B of DNA gyrase. This compound is a prenylated flavanone, also a group of flavonoids (Tronina et al., 2025). 6-Prenylnaringenin indicates antibacterial activity ranging from moderate to strong against Gram-positive and Gram-negative bacteria in vitro. It represents a predictive strong antibacterial activity compared to ciprofloxacin, a commonly used antibiotic (Tronina et al., 2025; Veiko et al., 2023).

Flavonoid, a known antioxidant agent, plays a crucial role as an antibacterial agent by disrupting bacterial enzymes such as helicases, DNA gyrase, and topoisomerases (in vitro study). Flavonoid-bacterial interaction affects bacterial growth, metabolism, and pathogenesis (Veiko et al., 2023). In addition, flavonoids can inhibit DNA gyrase and DNA supercoiling, facilitating transcription and replication process (Alhadrami et al., 2021; Ameji et al., 2025). 3',4'- Dimethoxy-7-hydroxyflavone, flavone group, is another flavonoid compound having predictive strong interaction to subunit B of DNA gyrase (ΔG = -8.5 kcal/mol) compared to ciprofloxacin. A flavonoid with a hydroxyl group shows greater antibacterial activity prediction (Shamsudin et al., 2022).

Sinapoyl malate belongs to the main esters of sinapic acid, which showed antimicrobial activity prediction (Mouterde et al., n.d.; Pham et al., 2020). In this study, those two compounds exhibited a predictive strong interaction with subunit B of DNA gyrase. Their binding affinity are higher than ciprofloxacin. Ciprofloxacin, fluoroquinolone-based, is a broad-spectrum antibiotic to treat *S. typhi* infection (Hasan et al., 2021). Ciprofloxacin-DNA gyrase complex is an indicated

antibacterial agent because this complex disrupts DNA replication in bacteria (Sharma et al., 2017).

The highest binding affinity of flavonoids (6-Prenylnaringenin) is related to the ability to bind the same catalytic residues as native, like Isoleucine (Ile) and Arginine (Arg) (Figure 1. 1A and 1B and Table 3). Ciprofloxacin binds to DNA gyrase subunit A using conventional hydrogen bonds and carbon-hydrogen bonds. 6-Prenylnaringenin binds to it using a conventional hydrogen bond only. The hydrogen bond makes the enzyme stable interaction with other molecules (Carlsson et al., 2018). SWISS Target Prediction on 6-Prenylnaringenin confirmed that around 26.7% target classes are enzymes. This prediction correlates with the ability of this compound as potent inhibitor to DNA gyrase subunit A and subunit B, one of *S. typhi* enzymes. The prediction is based on protein structure and the similarity principle (Daina et al., 2019).

Native ligand (ATP) and 6-Prenylnaringenin bind to the same catalytic residues as Valine (Val) and Isoleucine (Ile) on DNA gyrase subunit B (Figure 2. 2A and 2B and Table 3). Both ligands bind to DNA gyrase subunit B by conventional hydrogen bond, carbon hydrogen bond, and Alkyl. Hydrogen bond is very important in biological processes. It works in assigning protein-ligand selectivity and binding affinity. Hidrogen bond, the strongest type of bond of all, is formed between hydrogen atoms and highly electronegative atoms. In pharmaceutical context, the number of hydrogen bond donors and acceptors in a drug molecule significantly affect various chemical and physical properties such as melting point, water solubility, boiling point, chelation ability and acidity. Changes in these properties ultimately modulate the biological effects of the compound (Fadilaturahmah et al., 2023; Madushanka et al., 2023). This research is limited to the prediction antibacterial activity of Moringa oleifera leaves compounds in silico study only. Further study is needed to evaluate this activity in vitro and in vivo study until molecular confirmation.

5. CONCLUSION

M. oleifera, a nutrient rich plant, has various phytochemical compounds, that serve as potentially antibacterial activity to *S. typhi*. This study demonstrates that *M. oleifera* leaves methanolic extract is potent as an inhibitor of DNA gyrase of *S. typhi*. Phytochemical compounds binding to DNA gyrase subunit A were Naringenin, 6-Prenylnaringenin, 6-Methoxypodophyllotoxin, and Brefeldin A. Phytochemical compounds binding to DNA gyrase subunit B were Naringenin, 6-Prenylnaringenin, 3',4'-Dimethoxy-7-hydroxyflavone, Sinapoyl malate, and Malvidin. Those phytochemical compounds have a strong interaction with DNA gyrase of *S. typhi* compared to ciprofloxacin, a standard antibiotic. This study is limited to an in-silico study. Future research is needed to evaluate *M. oleifera* leaves extract on the DNA gyrase of *S. typhi* in vitro and in vivo methods to confirm the antibacterial activity.

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