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Computational Screening of Antibacterial Potential of Bioactive Compounds from *Rubia cordifolia* Roots and *Pongamia pinnata* Leaves

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Abstract: *Rubia cordifolia* and *Pongamia pinnata* has been traditionally used and well-documented medicinal plants known for their broad-spectrum antibacterial activity. DNA gyrase is an essential bacterial type II topoisomerase that introduces negative supercoils into DNA and is a validated target for antibacterial drug discovery. In the present *in silico* study, reported antibacterial constituents from *Rubia cordifolia* roots and *Pongamia pinnata* leaves were evaluated for their ability to bind the active site of DNA gyrase using molecular docking. Nine phytoconstituents (Alizarin, Purpurin, Munjistin, Rubiadin, Mollugin, Karanjin, Pongamol, Glabrin and Pinnatin) were docked into the gyrase binding pocket and scored for binding affinity and key intermolecular interactions. All compounds showed favourable docking poses and engaged the enzyme active site through hydrogen bonds, π–π stacking and hydrophobic contacts. The calculated binding energies ranged from –5.0 to –7.8 kcal·mol^{-1}, with Alizarin and Munjistin exhibiting the strongest predicted affinities (–7.8 kcal·mol^{-1}). Glabrin recorded the weakest score (–5.0 kcal·mol^{-1}), likely due to reduced complementarity with the binding pocket. Further molecular dynamics and *in vitro* studies warranted to confirm and expand the antibacterial potential upon these computational predictions.

Keywords: DNA gyrase, Alizarin, Munjistin, Antibacterial, *Pongamia pinnata*, *Rubia cordifolia*

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Introduction

Antibiotic resistance refers to the capacity of bacteria to withstand the effects of antimicrobial agents, such as penicillin or ciprofloxacin, which are intended to inhibit bacterial growth or induce cell death. This resistance compromises the effectiveness of standard treatments, making bacterial infections increasingly difficult to manage and significantly reducing available

therapeutic options (Ventola, 2015). Antibiotics are potent medications that have dramatically decreased the death rate from infectious infections that were formerly lethal. However, a number of negative consequences frequently accompany their effectiveness. Bacteria have developed inherent resistance mechanisms throughout time, usually as a result of structural or compositional changes that lessen the effectiveness of antibiotics. Protein synthesis pathways can be changed by genetic changes in bacterial DNA, resulting in the creation of modified cellular components and receptors that are resistant to antibiotics. Furthermore, the genomic architecture of bacterial populations that occupy the same ecological niche may be influenced by intrinsic genetic determinants of resistance, which would make antimicrobial treatment tactics much more challenging (Habboush and Guzman, 2018).

Antibiotics, even when prescribed and used properly, can cause antibiotic resistance at any moment. In actuality, when microorganisms change, antibiotic resistance may arise spontaneously. One factor contributing to the issue of antibiotic resistance is the abuse and overuse of antibiotics.

Naturally occurring products are abundant in a variety of active ingredients. Because of their strong pharmacological potential, these ingredients can be used to treat diseases effectively and with few or no negative side effects (Tiwari *et al.*, 2024). The secondary metabolites, such as alkaloids, flavonoids, lignins, terpenoids, steroids, glycosides, coumarins, and phenolic compounds, in plant materials produce the curative effect when they are used in the traditional medicinal practice (Mishra and Siddiqui, 2013). In Ayurveda, *Pongamia pinnata* (Karanja) and *Rubia cordifolia* (Manjistha), are well known for their wide range of ethnopharmacological uses. Historically, the plant's leaves, roots, seeds, and bark have all been used to treat a variety of conditions, including rheumatism, cough, ulcers, and skin conditions (Chopade *et al.*, 2008). Similarly, *R. cordifolia*, or Indian Madder, is highly regarded as a blood purifier and detoxifier due to its rich content of anthraquinones and other bioactive compounds (Divakar *et al.*, 2010).

Roots of *Rubia cordifolia* are rich in Purpurin, small amounts of xanthopurpurin, munjistin, and alizarin, pseudopurpurinmollugin, rubimallin, sitosterol and daucosterol (Tiwari *et al.*, 2024). Quercetin, kaempferol, pongamol, karanjin and flavone derivatives are the active constituents of the *Pongamia pinnata* leaves.

Molecular docking provides a logical and economical *in silico* method to examine the antibacterial potential of *Pongamia pinnata* and *Rubia cordifolia* in light of the growing threat of antimicrobial resistance. This technique is crucial because it forecasts the binding affinity between important bacterial proteins like DNA gyrase and phytochemicals found in plants, such as flavonoids and anthraquinones. Molecular docking finds the most promising candidates by virtually screening a large number of compounds. It also helps optimise lead compounds by elucidating their likely mechanism of action at the atomic level. In the end, this computational method offers a focused, hypothesis-driven framework that expedites the development of new antibacterial agents and confirms the conventional applications of these therapeutic plants (Bhagat *et al.*, 2021).

The objective of this study is to identify and validate potential antibacterial phytoconstituents from *Pongamia pinnata* and *Rubia cordifolia* by utilizing a molecular docking approach. This computational method will be used to predict the binding affinity and inhibitory potential of these plant-derived compounds against key bacterial targets, providing a foundation for future *in vitro* and *in vivo* studies to combat antimicrobial resistance.

Materials and Methods

Selection of Plant constituents

The pharmacological activities of *Rubia cordifolia* roots and *Pongamia pinnata* leaves have been

attributed to their rich phytochemical profile. *R. cordifolia* roots are a source of anthraquinones and naphthoquinones such as alizarin, purpurin, manjistin, rubiadin, and mollugin (Patil *et al*.,2009; Nyeem *et al*., 2018; Abebe *et al*., 2024). Similarly, *P. pinnata* leaves contain bioactive furano-flavonoids and flavones including karanjin, pongamol, pinnatin, and glabrin, reported to possess antibacterial, antifungal, and anti-inflammatory properties (Thakur *et al*., 2021; Rekha *et al*., 2021; Bhatt *et al*., 2025). Specifically, a key furanoflavanoid karanjin isolated from leaves has been shown to have antibacterial effect (Yasothkumar *et al*., 2023). These diverse phytoconstituents make both plants promising candidates for molecular docking-based antibacterial investigations.

Target bacterial proteins

To evaluate the antibacterial activity of selected constituents both Gram negative and Gram positive bacteria were selected. In Gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus pneumoniae*, targeting their Penicillin-Binding Proteins (PBPs) which are crucial for cell wall synthesis were selected and for Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* were used, with a focus on their essential enzymes like DNA gyrase and MurD ligase that are involved in DNA replication and cell wall synthesis, respectively.

Molecular docking

The molecular docking is a computational approach utilized to determine the interaction of phytoconstituents as ligand with the different proteins for their respective pharmacological potentials (Mustafa *et al*., 2023). As both the plants *Rubia cordifolia* and *Pongamia pinnata* reported to show antibacterial potential, the docking analysis using tools Biovia discovery studio, Open babel and Auto dock vina was performed between active constituents of plants and proteins of gram negative and gram positive bacteria to explore the antibacterial potential of plant (Asiamah *et al*., 2023). Firstly, Alizarin and purpurin from roots of *Rubia cordifolia* and karanjin and pongamol from leaves of *Pongamia pinnata* were selected and prepared for docking using open babel and auto dock software, prepared ligand were saved into PDBQT format. The crystal structure of receptor protein *E. coli* gyrase B (PDB ID: 6F86) was downloaded from protein data bank. By using biovia discovery studio protein was prepared in order to eliminate water molecules, adding polar hydrogen atoms and also elimination the ligand present after calculating the binding attributes, protein was converted in to PDBQT file in Auto dock tools. Auto dock vina was used to analyse the best pose between ligand and protein in terms of binding energy the more negative binding energy indicates the strog binding affinity between ligand and protein (Aliye *et al*., 2021).

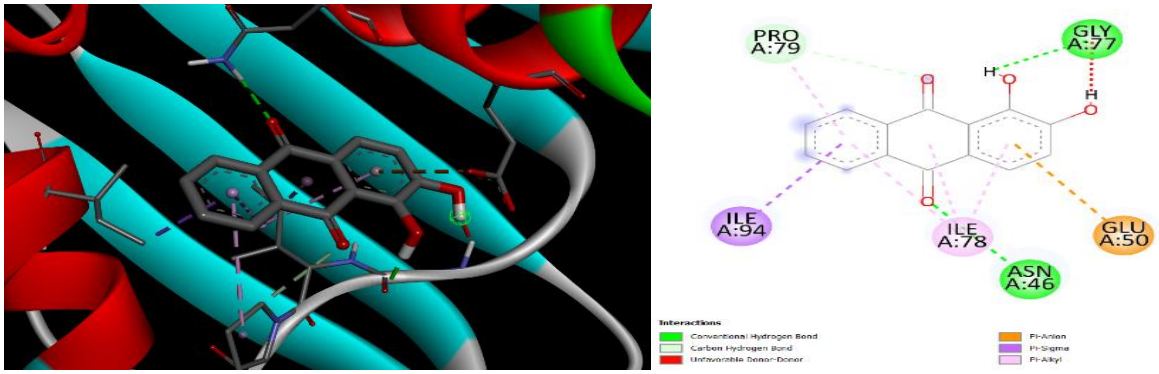
Results and Discussion

DNA gyrase enzyme is a critical target for antibacterial agents due to its essential role in DNA replication and bacterial survival. Since it it essential for survival, DNA gyrase is an excellent target for new antibacterial targets. In this view molecular docking between the reported constituents with the binding site of enzyme was performed. All the reported constituents show promising binding with the active site of enzyme targeting various amino acid residues. The resultant binding energy of all reported constituents was found between from –5.0 to –7.8 kcal·mol^{-1}, with Alizarin (Fig. 1) and Munjistin (Fig. 3) show highest binding affinities (–7.8 kcal·mol^{-1}) as depicted in Table 1.

The current study examined the binding interactions between the bacterial DNA gyrase enzyme, a verified molecular target essential for DNA replication and cell survival, and reported antibacterial phytoconstituents from *Rubia cordifolia* roots and *Pongamia pinnata* leaves. All of the tested compounds showed positive interactions with the active site residues, according to molecular docking results, confirming their potential as antibacterial agents.

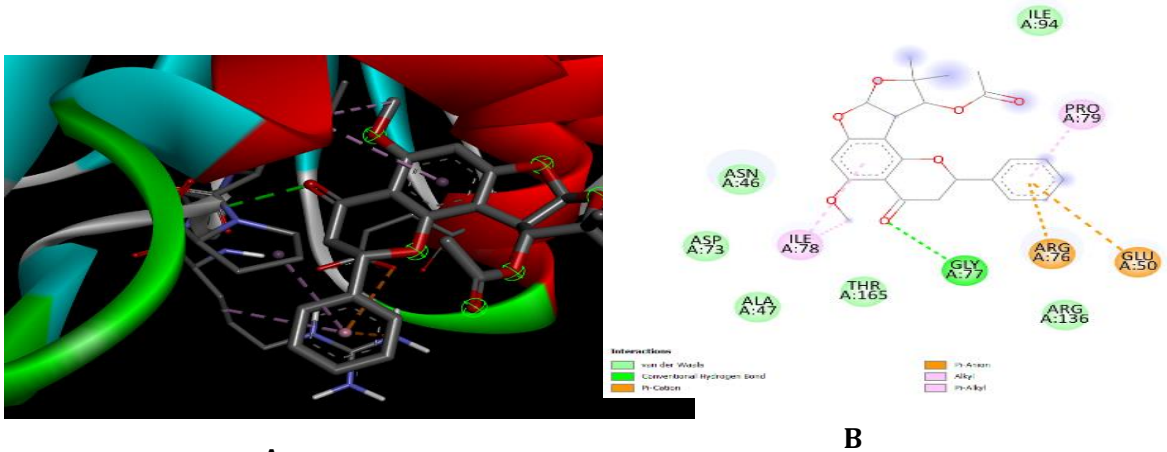
Table 1: Molecular docking analysis of active constituents from *Rubia cordifolia* roots and *Pongamia pinnata* leaves against DNA gyrase as a potential antibacterial target

Plants	Active constituents	Key interactions	Pub Chem ID	Binding affinity	Figure no.
Rubia cordifolia [Roots]	Alizarin	Pro79, Asn46, Ile78 and Ile94	80309	-7.8	1
	Purpurin	Ile78, Gly77, Asn46, Pro79, Glu50 and Arg76	44559427	-7.3	2
	Munjistin	Asp73, Asn46, Gly 77, Pro 79, Ile94, Glu50 and Ile78	160476	-7.8	3
	Rubiadin	Pro79, Ile94, Asn46 and Ile78	124062	-7.4	4
	Mollugin	Pro79, Asn46 and Ile78	124219	-6.8	5
Pongamia pinnata [Leaves]	Karanjin	Asp73, Asn46, Gly 77, Pro79,Ile94, Glu50 and Ile78	100633	-7.6	6
	Pongamol	Asn46,Ile94, Glu50, Asp49 and Ile78	689051	-6.7	7
	Glabrin	Gly,Pro79, Glu50 and Ile78	161850	-5.0	8
	Pinnatin	Gly77, Ala47, Thr165 and Ile78	5320607	-7.7	9



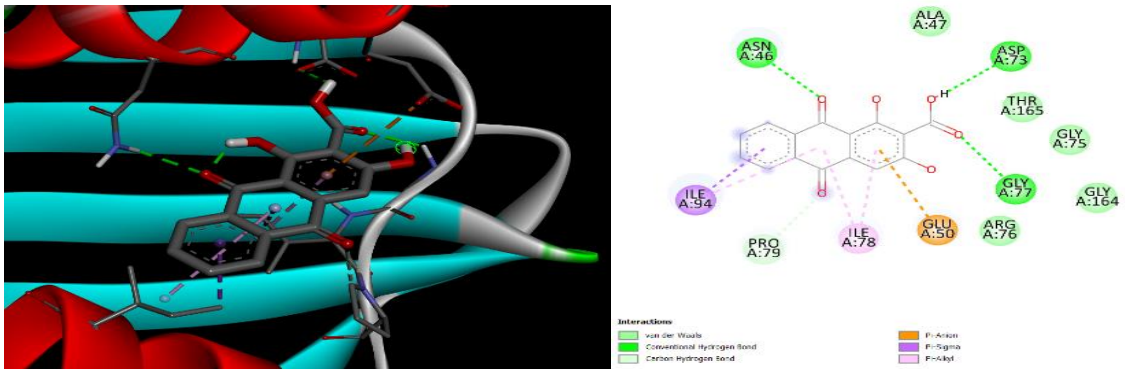
A **B**

Fig. 1: Binding interactions of Alizarin with DNA gyrase. (A) 3D binding pose of Alizarin within the DNA gyrase active site. (B) 2D interaction diagram showing hydrogen bonds with Gly77 and Asn46, carbon–hydrogen bond with Ile78, and additional π–π/π–alkyl interactions with Pro79, Glu50, and Ile94.



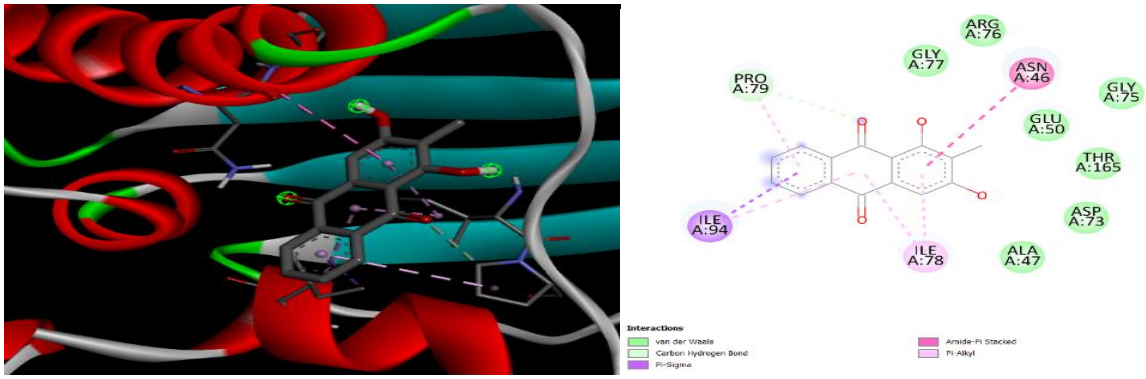
A **B**

Fig. 2: Binding interactions of Purpurin with DNA gyrase. (A) 3D binding pose of Purpurin within the DNA gyrase active site. (B) 2D interaction diagram showing hydrogen bonds with Gly77 and Asn46, carbon–hydrogen bond with Ile78, Pro 79 and additional π–π/π–alkyl interactions with Ile94, Glu50 and Arg 76.



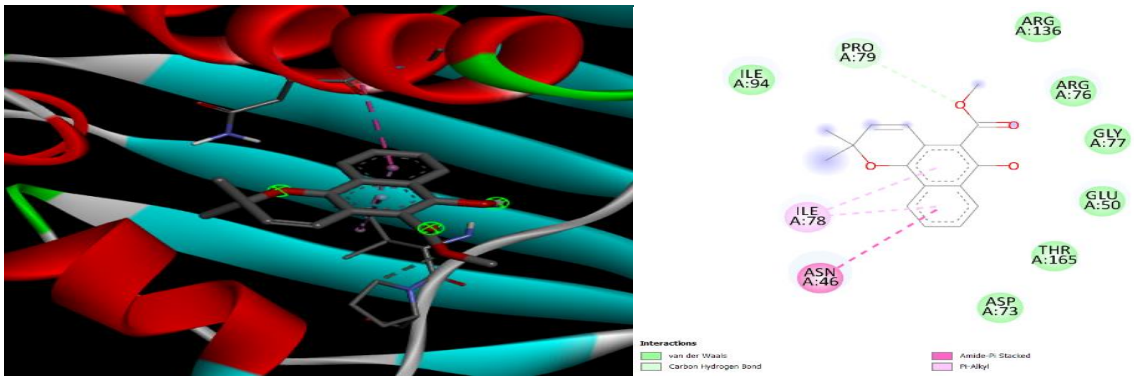
A **B**

Fig. 3: Binding interactions of Munjistin with DNA gyrase. (A) 3D binding pose of Munjistin within the DNA gyrase active site. (B) 2D interaction diagram showing hydrogen bonds with Asp73 and Asn46, Gly 77and carbon–hydrogen bond with Pro79 and additional π–π/π–alkyl interactions with Ile94, Glu50 and Ile78.



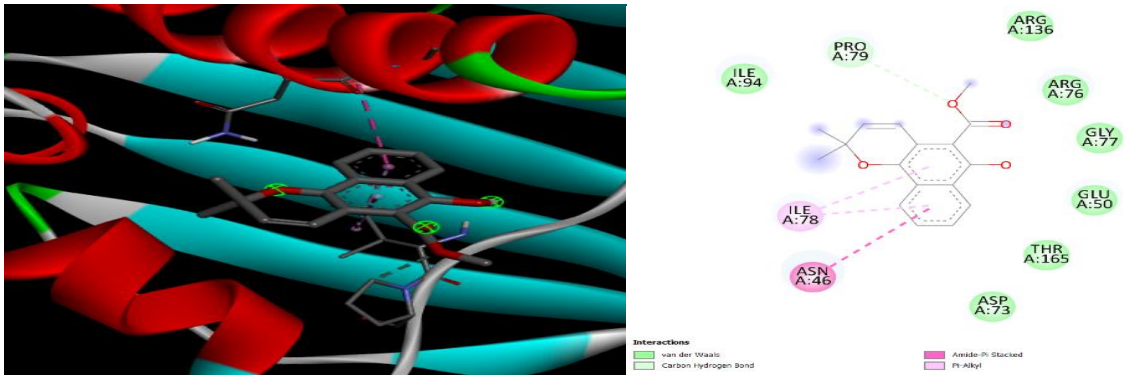
A **B**

Fig. 4: Binding interactions of Rubiadin with DNA gyrase. (A) 3D binding pose of Rubiadin within the DNA gyrase active site. (B) 2D interaction diagram showing carbon–hydrogen bond with Pro79 and additional π–π/π–alkyl interactions with Ile94, Asn46 and Ile78.



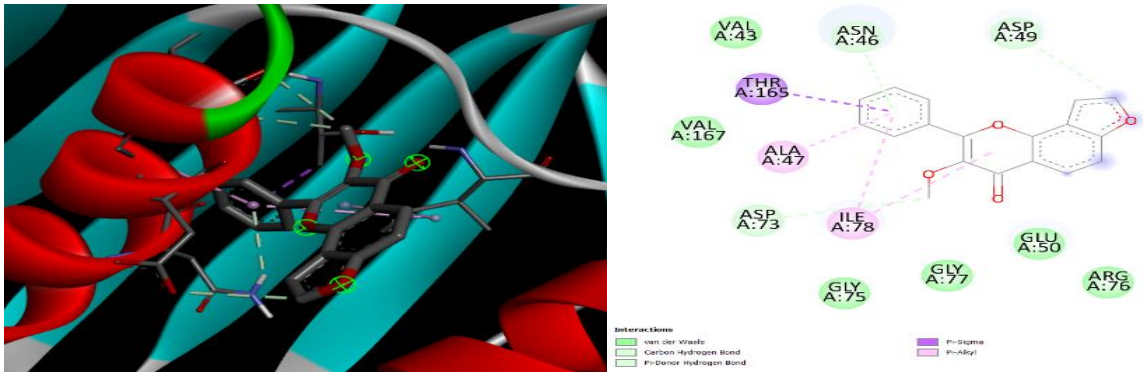
A **B**

Fig. 5: Binding interactions of Mollugin with DNA gyrase. (A) 3D binding pose of Mollugin within the DNA gyrase active site. (B) 2D interaction diagram showing carbon–hydrogen bond with Pro79 and additional π–π/π–alkyl interactions with Asn46 and Ile78.



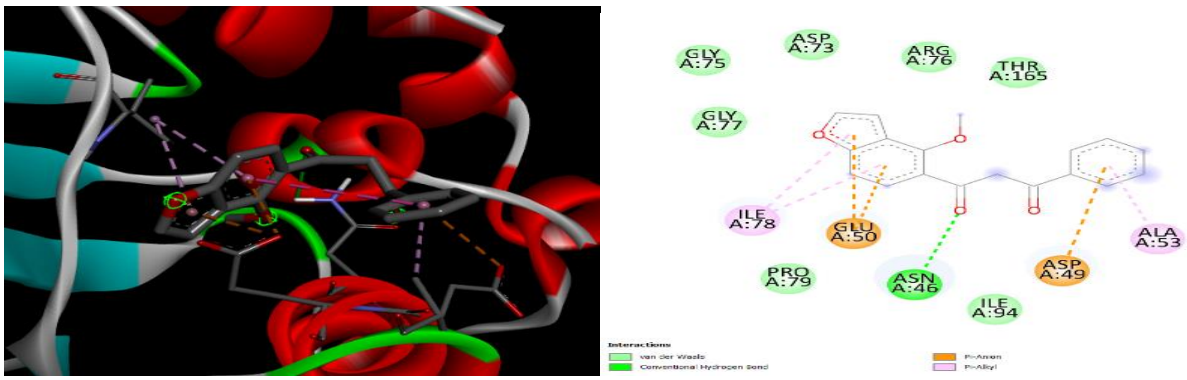
A **B**

Fig. 5: Binding interactions of Mollugin with DNA gyrase. (A) 3D binding pose of Mollugin within the DNA gyrase active site. (B) 2D interaction diagram showing carbon–hydrogen bond with Pro79 and additional π–π/π–alkyl interactions with Asn46 and Ile78.



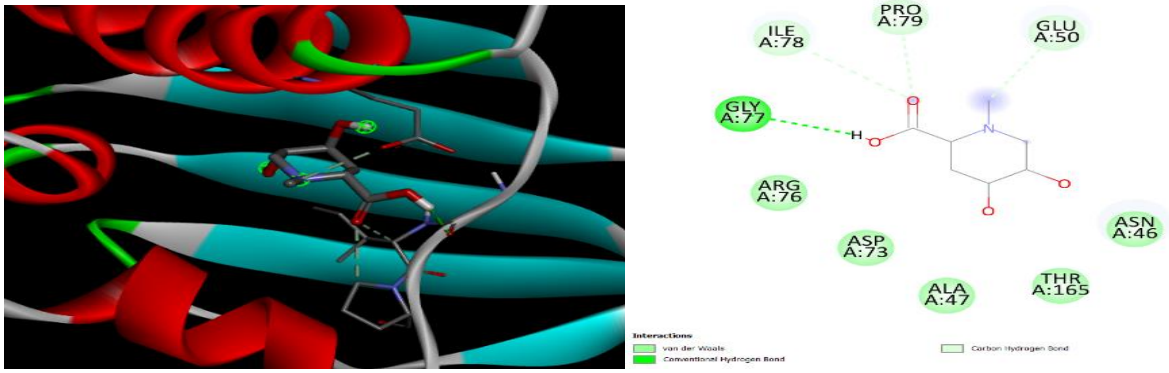
A **B**

Fig. 6: Binding interactions of Karanjin with DNA gyrase. (A) 3D binding pose of karanjin within the DNA gyrase active site. (B) 2D interaction diagram showing hydrogen bonds with Asp73 and Asn46, Gly 77, Pro 79 and carbon–hydrogen bond with Pro79 and additional π–π/π–alkyl interactions with Ile94, Glu50 and Ile78.



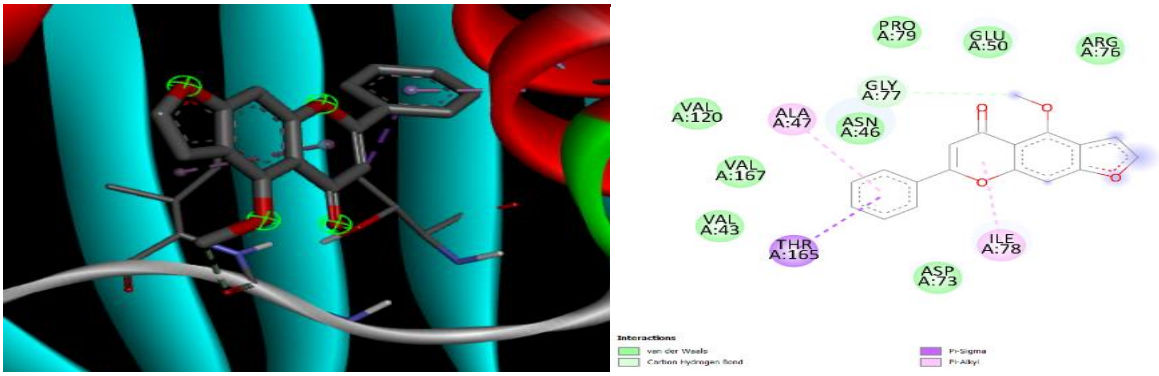
A **B**

Fig. 7: Binding interactions of Pongamol with DNA gyrase. (A) 3D binding pose of Pongamol within the DNA gyrase active site. (B) 2D interaction diagram showing hydrogen bonds with Asn46and additional π–π/π–alkyl interactions with Ile94, Glu50, Asp49 and Ile78.



A B

Fig. 8: Binding interactions of Glabrin with DNA gyrase. (A) 3D binding pose of Glabrinwithin the DNA gyrase active site. (B) 2D interaction diagram showing hydrogen bonds with Gly 77and carbon–hydrogen bond with Pro79, Glu50 and Ile78.



A B

Fig. 9: Binding interactions of Pinnatin with DNA gyrase. (A) 3D binding pose of Pinnatin within the DNA gyrase active site. (B) 2D interaction diagram showing carbon–hydrogen bond with Gly77 and additional π–π/π–alkyl interactions with Ala47, Thr165 and Ile78.

Alizarin and Munjistin formed numerous hydrogen bonds and hydrophobic contacts with residues like Pro79, Asn46, Ile78, and Ile94, demonstrating the strongest binding affinities (–7.8 kcal/mol) among the constituents of *Rubia cordifolia*. Purpurin formed extra hydrogen bonds with Arg76 and Glu50, indicating that hydroxyl substituents on anthraquinone structures improve target complementarity. Rubiadin and Purpurin (Figs. 2, 4) also showed strong binding affinities (-7.4 and -7.3 kcal/mol, respectively). Mollugin, (Fig. 5) on the other hand, showed a comparatively lower affinity (–6.8 kcal/mol), most likely as a result of fewer polar interactions.

Notable activity was also shown by the phytoconstituents found in *Pongamia pinnata* leaves. Strong affinities were demonstrated by pinnatin (Fig. 9) (-7.7 kcal/mol) and karanjin (Fig. 6) (-7.6 kcal/mol), which interacted with important residues such as Asp73, Asn46, Gly77, Pro79, and Glu50. With interactions involving Asn46, Glu50, and Ile78, ponagamol (Fig. 7) demonstrated a moderate binding affinity (–6.7 kcal/mol). Glabrin (Fig. 8) on the other hand, was the weakest binder (-5.0 kcal/mol), most likely as a result of its less than ideal orientation and decreased hydrogen bonding ability.

The docking results highlight anthraquinone derivatives from *R. cordifolia* (Alizarin, Munjistin, Rubiadin) and flavonoids from *P. pinnata* (Pinnatin, Karanjin) as the most promising candidates for DNA gyrase inhibition. The consistency of their interactions with conserved catalytic residues such as Asn46, Gly77, Ile78, and Pro79 suggests a possible mechanism of antibacterial activity through disruption of DNA

supercoiling. Importantly, the predicted affinities of these compounds fall within the range of reported natural inhibitors of DNA gyrase, underscoring their therapeutic relevance.

While these *in silico* findings provide valuable insights, limitations inherent to docking studies must be acknowledged. Docking does not fully account for protein flexibility, solvent effects, or kinetic parameters. Therefore, future studies should include molecular dynamics simulations to validate pose stability and free-energy calculations for more accurate binding predictions. Furthermore, *in vitro* and *in vivo* assays will be necessary to confirm DNA gyrase inhibition and antibacterial efficacy, as well as to evaluate cytotoxicity and pharmacokinetic profiles.

In conclusion, this study supports the antibacterial potential of bioactive phytoconstituents from *R. cordifolia* and *P. pinnata* through DNA gyrase inhibition, with Alizarin, Munjistin, and Pinnatin emerging as lead scaffolds for further optimization.

Conclusion

This *in silico* study demonstrated that bioactive constituents from *Rubia cordifolia* roots and *Pongamia pinnata* leaves exhibit promising binding affinities toward DNA gyrase, a critical antibacterial target. Among them, Alizarin, Munjistin, and Pinnatin showed the strongest interactions with key active-site residues, suggesting their potential as lead compounds for novel antibacterial drug development. These findings provide a molecular basis for the traditional use of these plants and warrant further validation through molecular dynamics simulations and experimental studies.

Ethical Statement

No animal has been used or sacrificed for this study, hence Ethical Approval not required.

Author Contributions

Singh Prasant Kumar: contributed to the methodology development; *Srivastava Mohit*: performed computational screening, data analysis, and contributed to results interpretation; *Mishra Bharat*: reviewed the manuscript critically for important intellectual content and helped in final proofing and formatting; *Tiwari Archita*: assisted in literature review, compound selection, docking analysis, and manuscript drafting. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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