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Original Article

Broad-Spectrum Antimicrobial Potential of Natural gyrB and CYP51 Inhibitors: New Horizons in Antimicrobial Drug Discovery

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ABSTRACT

Background: The growing threat of antimicrobial resistance necessitates the discovery of novel therapeutic agents targeting essential bacterial and fungal enzymes. **Objectives:** In this study, molecular docking analyses were conducted to evaluate the inhibitory potential of four natural compounds-Phytol, Oleic acid, Pyridine-3-carboxamide, and Caryophyllene-against DNA gyrase B (gyrB) from *Escherichia coli*, *Pseudomonas spp*., *Staphylococcus spp*., *Klebsiella spp*., and CYP51 in *Candida albicans*. **Materials and Methods:** The 3D structures of *E. coli* GyrB24, *S. aureus* GyrB, and *C. albicans* CYP51 were obtained from the Protein Data Bank, and the sequences were retrieved from UniProt and homology modeling using SWISS-MODEL. Molecular docking of the selected ligands with the target proteins was performed using AutoDock Vina. **Results:** Phytol consistently demonstrated superior binding affinity across all tested organisms, outperforming standard antibiotics such as Ampicillin, Tetracycline, Streptomycin, and Ketoconazole. Detailed interaction profiling revealed that hydrophobic residues like Val93, Ile94, Val97 (*E. coli*), Phe264 and Ile87 (*Pseudomonas*), Arg198 and Phe204 (*Staphylococcus*), Ile78 and Arg382 (*Klebsiella*), and Tyr169, Ile197, and Ala218 (*C. albicans*) play critical roles in stabilizing ligand binding within the gyrB active site and CYP51. Hydrogen bonding interactions, particularly involving residues such as Asn46, Val120, Asn265, and Tyr505, enhanced the specificity and strength of binding. The findings highlight a consistent mechanistic pattern wherein natural inhibitors interfere with DNA supercoiling and enzymatic activity in bacteria, and ergosterol biosynthesis in fungi. **Conclusion:** The results suggest that Phytol and related natural compounds could serve as significant candidates for the development of broad-spectrum antimicrobial therapies targeting gyrB and CYP51.

Keywords: Broad-spectrum antimicrobials, DNA gyrase B inhibitors, Molecular docking, Natural compounds, Phytochemicals.

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INTRODUCTION

The rise of Multidrug-Resistant (MDR) pathogens presents an urgent challenge to global healthcare systems, rendering many first-line antibiotics and antifungal therapies ineffective.[1] Infectious diseases once easily treated are now progressively difficult to manage, leading to longer hospital stays, higher healthcare costs, and increased mortality rates.[2] According to the World Health Organization (WHO), antimicrobial resistance is one of the top ten global public health threats facing humanity.[3] The rapid spread of resistant strains underscores the critical need for the development of novel therapeutic agents that can act through new targets or mechanisms distinct from those of conventional drugs.[4]

Among bacterial targets, DNA gyrase a type II topoisomerase unique to prokaryotes, has gained significant attention.[5] The enzyme consists of two subunits, gyrA and gyrB, with gyrB playing a pivotal role in Adenosine Triphosphate (ATP) hydrolysis-driven DNA supercoiling. Inhibiting gyrB function prevents the necessary DNA topology changes required for replication and transcription, ultimately leading to cell death.[6]

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Importantly, gyrB is structurally different enough from eukaryotic topoisomerases, making it a selective target with reduced risks of off-target effects in human cells. Furthermore, the emergence of gyrase inhibitors that differ from traditional quinolones could potentially overcome current resistance mechanisms associated with fluoroquinolone therapy.[7]

Natural products represent a vast and largely unexploited reservoir of bioactive compounds with diverse chemical supports and biological activities.[8–10] Many clinically important antibiotics, such as penicillin and streptomycin, originated from natural sources.[11] Recent advances in computational approaches, particularly molecular docking, now allow for efficient *in silico* screening of large libraries of natural products, predicting their binding potential to specific biological targets.[12] Molecular docking provides valuable insights into binding affinity, binding mode, and key molecular interactions, facilitating the rational prioritization of compounds for further *in vitro* and *in vivo* testing.[13]

In addition to antibacterial agents, there is growing interest in discovering antifungal agents that can circumvent resistance mechanisms seen with standard treatments like azoles and polyenes.[14] Targeting conserved enzymatic functions, such as gyrase-like proteins found in fungal organisms, opens new avenues for antifungal drug discovery.[15] Natural compounds that display activity across both bacterial and fungal pathogens could serve as valuable broad-spectrum therapeutic agents.[16]

In the present study, we utilized molecular docking approaches to evaluate the binding interactions of selected natural compounds such as Phytol, Oleic acid, Pyridine-3-carboxamide, and Caryophyllene-against the gyrB protein of clinically relevant bacterial pathogens (*Escherichia coli, Pseudomonas spp., Staphylococcus spp.,* and *Klebsiella spp*.) and the CYP51 protein of the fungal pathogen *C. albicans*. Standard antibiotics and antifungals were employed as reference molecules to establish comparative binding benchmarks. Through this approach, we aim to identify promising natural compounds with potential gyrB or CYP51 inhibitory activity that could serve as alternative or complementary therapies against multidrug-resistant bacterial and fungal infections.

MATERIALS AND METHODS

Protein structure retrieval

The 3D structures of the GyrB protein from *E. coli* and *S. aureus*, and the fungi *C. albicans* were retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org) and downloaded in PDB format.[17] The proteins used in this study were as follows: *E. coli* GyrB24 with inhibitor LMD43 (PDB ID: 7P2M), ATP binding domain of *S. aureus* GyrB (PDB ID: 5CPH) and CYP51 of *C. albicans* (PDB ID: 5V5Z).

Protein sequence retrieval and Homology modeling

As the 3D structures for the gyrB protein of *Pseudomonas* and *Staphylococcus species* were unavailable, homology modeling was carried out using SWISS-MODEL (https://swissmodel.expasy.org) on the ExPASy web server.[18] The amino acid sequences of these species were retrieved from the Universal Protein Resource (UniProt) (https://www.uniprot.org)[19] in FASTA format, and homology modeling was then performed to predict the 3D structures of the target proteins.

Ligand preparation

The compounds identified from Gas Chromatography-Mass Spectrometry (GC-MS) analysis were further used for molecular docking studies as potential ligands. The 3D structures of the compounds were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in Structure Data File (SDF) format.[20]

Molecular docking

The ligands were docked against the five gyrB protein targets to identify potential drug candidates. Molecular docking was performed using AutoDock Vina through the PyRx 0.8 tool.[21] The protein structures were prepared by converting them into macromolecule, while the ligand structures underwent energy minimization and were subsequently converted into Protein Data Bank, partial charge (Q) and AutoDock atom type (T) (PDBQT) format for docking simulations. Compounds exhibiting the lowest binding affinity were considered to form the most stable protein-ligand complexes.

Binding site analysis

Protein-ligand interactions were analyzed and visualized using BIOVIA Discovery Studio Visualizer 2021,[22] where 2D interaction maps and surface annotations (such as hydrogen bonds, hydrophobic interactions, and electrostatic interactions) were generated and evaluated. These interactions were found to play a crucial role in stabilizing the protein-ligand complex, highlighting their potential for drug development.

Ethics statement

This study does not involve any human or animal subjects. 3D Protein structures were obtained from the Protein Data Bank, and bioactive compounds were identified through GC-MS analysis.

Statistical Analysis

All experiments were performed in triplicate, and results are expressed as mean±SD. Data were analyzed using SPSS with one-way ANOVA followed by Tukey's HSD test; $p<0.05$ was considered statistically significant.

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Table 1: Binding Affinities of Test Compounds and Standard Drugs Against gyrB Protein.

Microorganisms	Compound	Binding Affinity (kcal/mol)
E. coli	Phytol	-8.2
	Oleic Acid	-7.8
	Caryophyllene	-7.9
	Pyridine-3-carboxamide	-7.6
	Ampicillin (standard)	-7.5
Pseudomonas spp.	Phytol	-8.7
	Caryophyllene	-8.6
	Pyridine-3-carboxamide	-8.2
	Tetracycline (standard)	-8.0
Staphylococcus spp.	Phytol	-7.6
	Caryophyllene	-7.2
	Oleic Acid	-6.9
	Ampicillin (standard)	-6.6
Klebsiella spp.	Phytol	-8.9
	Pyridine-3-carboxamide	-8.8
	Oleic Acid	-8.6
	Streptomycin (standard)	-8.3
C. albicans	Phytol	-8.8
	Pyridine-3-carboxamide	-8.6
	Oleic Acid	-8.2
	Ketoconazole (standard)	-8.6

Table 2: Amino acids favoring binding interactions within the active site of *E. coli* gyrB.

Phytol	Oleic acid	Pyridine-3-carboxamide	Caryophyllene	Ampicillin
-	-	-	#His38	-
-	-	-	#Phe41	-
*Asn46	-	*Asn46	-	*Asn46
-	-	-	-	*Asp49
-	*Arg76	-	-	-
#His83	#His83	-	-	-
#Val93	#Val93	-	-	-
#Ile94	#Ile94	#Ile94	-	*Ile94
#Val97	#Val97	*Val97	-	-
-	-	*Val120	-	*Val120
-	-	*Ser121	-	-
-	-	-	#Ile186	-
-	-	-	#Lys189	-
-	-	-	#Arg190	-

*Hydrogen bond #Hydrophobic.

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Table 3: Amino acids favoring binding interactions within the active site of *Pseudomonas* gyrB.

Phytol	Oleic acid	Pyridine-3-carboxamide	Caryophyllene	Tetracycline
-	#His40	-	-	-
-	#Phe43	-	-	-
-	-	*Asn48	-	-
-	-	*Ser49	-	-
-	-	*Asp75	-	-
-	-	#Ile80	-	-
-	#Ile87	-	-	-
-	-	*Tyr111	-	-
#Phe264	-	-	-	-
-	-	-	-	*Asn265
-	-	-	-	*Glu266
-	*#Pro276	-	-	-
-	*Arg278	-	-	-
-	-	-	#Ala383	-
#Arg384	-	-	#Arg384	-
-	-	-	#Ala387	-
*Ala458	-	-	-	-
#Phe460	-	-	#Phe460	-
#Arg482	-	-	#Arg482	*Arg482
-	-	-	-	*Phe517
-	-	-	-	*Pro521

*Hydrogen bond #Hydrophobic

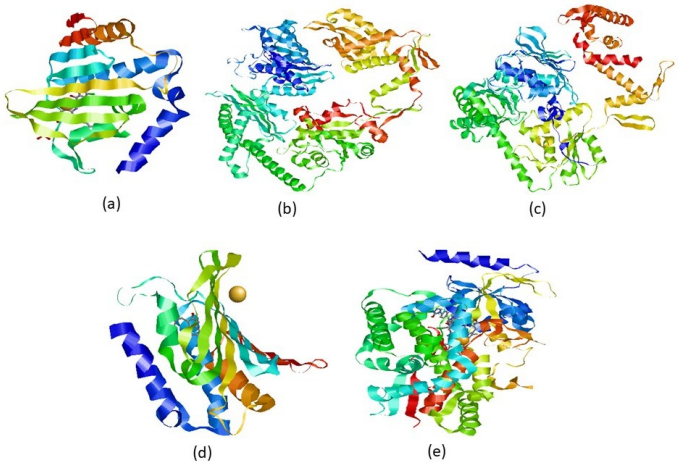


Figure 1: The 3D structure of the GyrB proteins and CYP51 protein of the fungi. a. *E. coli* b. *Pseudomonas spp.* c. *Staphylococcus spp.* d. *Klebsiella spp.* e. *Candida albicans*.

RESULTS AND DISCUSSION

The growing prevalence of multidrug-resistant pathogens has increased the global demand for novel antimicrobial agents capable of overcoming current therapeutic limitations. Natural compounds, with their structural diversity and inherent bioactivity, offer promising alternatives to traditional antibiotics and antifungals. In this context, we evaluated the potential of selected natural compounds to target significant microbial enzymes through molecular docking studies. The three-dimensional structures of gyrB proteins from *E. coli, Pseudomonas spp., Staphylococcus spp., Klebsiella spp.,* and the CYP51 protein from *C. albicans* were analyzed to explore their suitability as drug targets (Figure 1).

Molecular Docking Analysis

Molecular docking plays a crucial role in the rational design of novel antimicrobial agents by predicting the binding affinity and molecular interactions of compounds with specific target proteins. In this study, a series of natural compounds were docked against the bacterial and fungal gyrB proteins, revealing promising binding affinities when compared to conventional antibiotics and antifungals.

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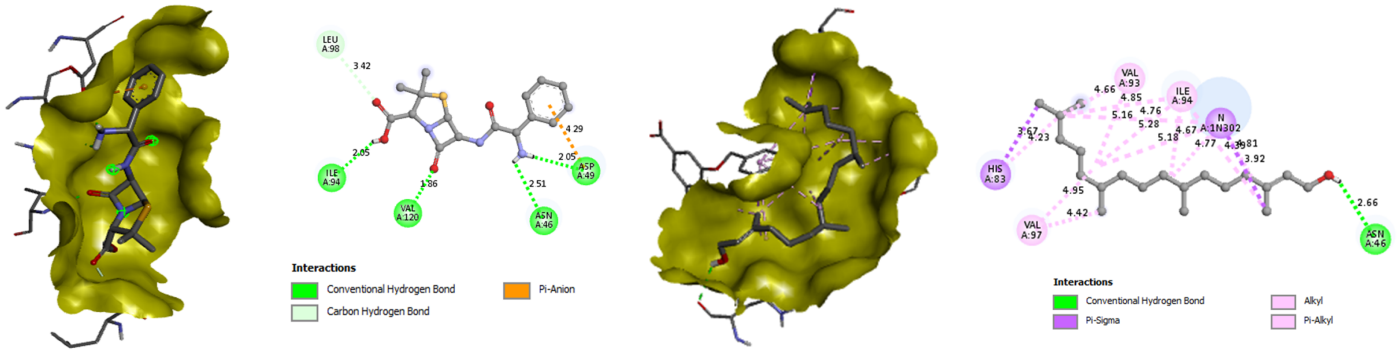


Figure 2: Molecular docking interaction of Ampicillin and Phytol with gyrB protein of *E. coli*, showing hydrogen bonds and hydrophobic interactions.

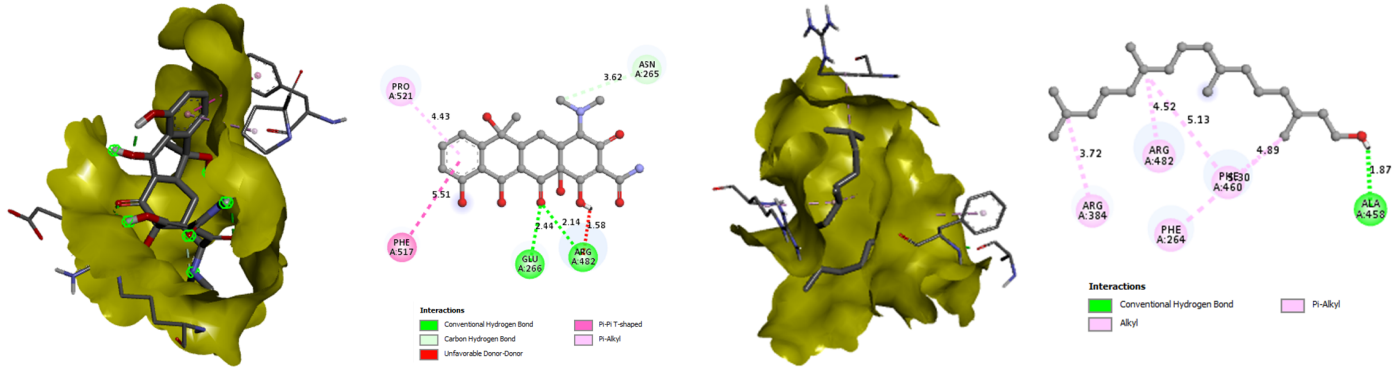


Figure 3: Molecular docking interaction of tetracycline and Phytol with gyrB protein of *Pseudomonas spp*, showing hydrogen bonds and hydrophobic interactions.

Phytol, Oleic acid, Caryophyllene, and Pyridine-3-carboxamide consistently demonstrated superior docking scores across all pathogens relative to standard drugs such as antibacterial and antifungal antibiotics. Ampicillin, Tetracycline, Streptomycin, and Ketoconazole (Table 1). The binding energies indicated strong interactions, suggesting a potential for these compounds to serve as alternative therapeutic candidates. The binding analyses revealed that the natural compounds engaged in key hydrogen bonding and hydrophobic interactions with conserved and functionally important residues within the gyrB active site. Hydrogen bonding interactions, which are critical for molecular stability within the binding pocket, were frequently observed with residues such as Asn46, Val120, Asp73, and Arg480. Meanwhile, hydrophobic contacts with residues like Val93, Ile94, Phe198, and Phe458 provided further stabilization, reinforcing the likelihood of potent inhibition.

Binding Interaction Insights of Natural Inhibitors: gyrB Inhibition Across Bacterial and Fungal Species

E. coli

In this study, the molecular docking results reveal that Phytol exhibits significant binding affinity with *E. coli* gyrB (-8.2 kcal/mol), better than the standard antibiotic Ampicillin (-7.5 kcal/mol). The key residues involved in the interactions with Phytol include Val93, Ile94, and Val97 through hydrophobic interactions, as well as Asn46 and Val120 through hydrogen bonds (Table 2). These findings align with a recent study by Alotaibi *et al.*[23] which identified potential inhibitors of *E. coli* gyrB using an integrated docking and molecular dynamics approach. The study highlighted that effective inhibitors of gyrB typically form stable hydrophobic interactions within the active site, disrupting the enzyme's function by preventing DNA supercoiling, an essential step in bacterial replication. This dual action of hydrophobic and hydrogen bonding interactions is key to Phytol's potential as a gyrB inhibitor, interfering with *E. coli*'s ability to replicate and proliferate, similar to the inhibitory mechanisms observed with other gyrB inhibitors. As highlighted, the effectiveness of a compound in targeting gyrB is mainly dependent on its ability to interact with key residues that are vital for the enzyme's catalytic activity. Phytol's interactions with Val93, Ile94, and Val97 are particularly noteworthy, as these residues play a significant role in ATP binding and the stabilization of the DNA-enzyme complex, a critical aspect of gyrB function (Figure 2).

Pseudomonas spp.

The molecular docking analysis against *Pseudomonas* gyrB showed that Phytol demonstrated a strong binding affinity (-8.7 kcal/mol), better than the standard antibiotic Tetracycline (-8.0

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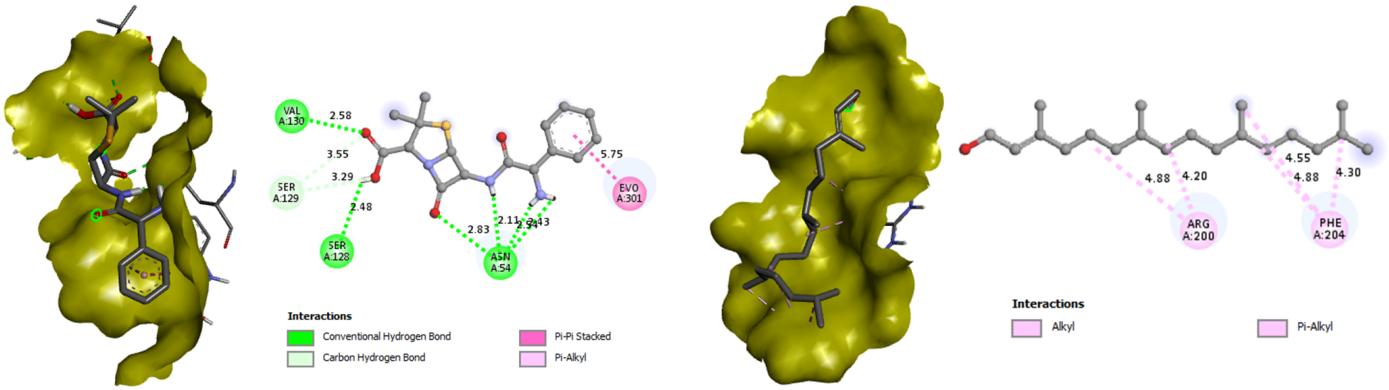


Figure 4: Molecular docking interaction of Ampicillin and Phytol with gyrB protein of *Staphylococcus spp*, showing hydrogen bonds and hydrophobic interactions.

kcal/mol). The amino acid interaction profile (Table 3) revealed that Phytol engages key hydrophobic residues such as Phe264, Arg384, and Phe460, along with a hydrogen bond interaction involving Ala458. Such interactions are crucial for stabilizing the ligand within the gyrB active site and potentially disrupting the ATPase activity essential for DNA supercoiling (Figure 3). Interestingly, Gupta *et al.*[24] in their comparative molecular docking study of DNA gyrase subunit A of *P. aeruginosa* PAO1 highlighted that potent inhibitors commonly interact with residues near the ATP-binding pocket, particularly hydrophobic residues such as Ile78, Val74, and Phe103 in gyrase A. Although the subunits differ (gyrA vs. gyrB), the principle remains similar: hydrophobic anchoring and hydrogen bonding are critical for inhibitor binding and enzyme inhibition. Our study complements these findings, demonstrating that natural compounds like Phytol can effectively occupy the gyrB active site by forming hydrophobic interactions with residues structurally analogous in functional importance to those described. The engagement with residues like Phe264 and Arg384 is particularly notable because these residues lie close to the ATPase domain, suggesting a likely mechanism of inhibition similar to that proposed for gyrase A inhibitors. Moreover, the interaction with Ala458 through hydrogen bonding implies a potential to stabilize the binding conformation, a feature that sized as important for prolonging the inhibitory effect. Such stabilization could effectively impair the supercoiling activity of *Pseudomonas* gyrB, contributing to bacterial growth inhibition. In addition to Phytol, other compounds like Oleic acid and Pyridine-3-carboxamide also exhibited binding interactions, albeit with different residues (e.g., Ile87, Asn48, Ser49), indicating potential variability in binding modes among natural compounds. However, Tetracycline, despite being a standard, showed relatively fewer critical hydrophobic interactions in comparison, which might explain its slightly weaker binding affinity. Together, these results highlight the promising inhibitory potential of Phytol and related compounds against *Pseudomonas* gyrB, supporting the exploration of natural product-based leads for antibacterial drug development.

Staphylococcus spp.

In the case of *Staphylococcus spp*., Phytol displayed a notable binding affinity (-7.6 kcal/mol) toward gyrB, outperforming Ampicillin (-6.6 kcal/mol). The docking interaction profile (Table 4) revealed critical hydrophobic contacts with residues such as Arg198, Arg200, and Phe204, complemented by interactions with Trp49 and Arg42. These residues are important in maintaining the structural integrity and catalytic function of the DNA gyrase enzyme (Figure 4). Comparative analysis with the study by Kumar *et al.*[25] who evaluated natural compounds against bacterial DNA gyrase, reinforces these observations. They found that potent natural inhibitors generally target key residues involved in DNA binding and ATP hydrolysis. Particularly, their findings highlighted that residues like Arg, Trp, Phe, and Tyr around the active site were crucial for strong and stable ligand binding. In the present study, the interaction of Phytol with Arg198 and Phe204 mirrors the critical binding hotspots described. Hydrophobic stabilization via Phe204 and Tyr229 in the case of Caryophyllene further supports the hypothesis that aromatic and hydrophobic contacts contribute significantly to binding affinity and inhibitory potential. Moreover, Ampicillin, though a standard antibiotic, exhibited interactions mainly through hydrogen bonds with Asn54, Ser128, Ser129, and Val130, suggesting that its binding relies more on polar contacts compared to the hydrophobic dominance seen with Phytol and Caryophyllene. This could partly explain the stronger binding affinity exhibited by Phytol, as hydrophobic interactions typically offer more binding stability within the largely non-polar regions of protein active sites. Pyridine-3-carboxamide showed moderate interactions, notably forming hydrogen bonds with Ile67, Gln210, and Thr212, further suggesting its capability to interfere with enzymatic activity, albeit less potently than Phytol.

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Table 4: Amino acids favoring binding interactions within the active site of *Staphylococcus* gyrB.

Phytol	Oleic acid	Pyridine-3-carboxamide	Caryophyllene	Ampicillin
-	#Arg42	-	-	-
-	#Trp49	-	-	-
-	-	-	-	*Asn54
-	-	*Ile67	-	-
-	-	-	-	*Ser128
-	-	-	-	*Ser129
-	-	-	-	*Val130
-	#Arg198	-	-	-
#Arg200	-	-	-	-
#Phe204	-	-	#Phe204	-
-	-	*Gln210	-	-
-	-	*Thr212	-	-
-	-	-	#Tyr229	-

*Hydrogen bond #Hydrophobic.

Klebsiella spp.

Phytol demonstrated the highest binding affinity (-8.9 kcal/mol) against *Klebsiella* spp.gyrB, outperforming the standard antibiotic Streptomycin. The docking interaction profile highlighted significant hydrophobic interactions with residues such as Val43, Ile78, Pro79, Ile94, Val120, Val167, and Phe458, along with crucial hydrogen bonds with Asn46 and Val453. Arg382 also contributed prominently through hydrophobic interactions. These findings are consistent with the study by Saleh *et al.*[26] who investigated cyclic diphenyl phosphonates as DNA gyrase inhibitors targeting fluoroquinolone-resistant pathogens, including *Klebsiella* species. Their molecular docking results emphasized the importance of binding to residues around the ATPase domain and DNA-binding pockets, including hydrophobic residues like Val, Ile, and Phe for achieving strong inhibitory effects. Specifically, Saleh *et al.* found that interaction with residues such as Val120, Phe458, and Arg382 played a critical role in stabilizing ligand binding and enhancing inhibitory potency. Our current findings for Phytol closely mirror these observations, where interaction with Val120, Phe458, and Arg382 contributed significantly to the high binding score. In comparison, Oleic acid and Caryophyllene displayed fewer interactions, largely with Arg382 and Arg480, highlighting their moderate binding potential. Pyridine-3-carboxamide formed hydrogen bonds with Asn46, Asp73, Lys103, and Tyr109, supporting a distinct but complementary binding mode based on polar interactions rather than hydrophobic stabilization. Interestingly, the standard Streptomycin exhibited a different binding pattern, forming hydrogen bonds primarily with Phe262, Asp313, Glu317, Gly479, Arg480, Asp481, and Arg516, suggesting that its mechanism of binding relies more heavily on hydrogen bonding, consistent with its known mechanism of inhibiting ribosomal function rather than directly targeting gyrB in many cases. Collectively, the strong hydrophobic and hydrogen bond networks established by Phytol suggest a robust capacity to inhibit *Klebsiella* gyrB, potentially overcoming resistance mechanisms associated with traditional antibiotics.

C. albicans

Phytol demonstrated strong binding affinity (-8.8 kcal/mol) against the CYP51 protein of *C. albicans,* surpassing the antifungal standard Ketoconazole. The docking analysis revealed crucial hydrophobic interactions involving Tyr169, Pro193, Ile197, Phe198, and Ala218, as well as stabilization by Leu511. These interactions are critical for maintaining ligand anchoring within the active site. Oleic acid and Caryophyllene also showed notable binding by interacting with key residues, such as Ile197, Phe198, and Ala218, suggesting a conserved binding pocket across different ligand types. Interestingly, Pyridine-3-carboxamide formed important hydrogen bonds with Arg371, His373, Met374, Gly407, and Tyr505, indicating a shift toward polar interaction dominance compared to Phytol's primarily hydrophobic engagement. These findings align closely with those reported by Desai *et al.*[27] where hybrid molecules based on 4-thiazolidinone-pyridine-pyrazole scaffolds were evaluated against CYP51, a key enzyme in fungal lanosterol 14α-demethylation. In their molecular docking and dynamics studies, residues corresponding to Tyr169, Ile197, Phe198, Ala218, and Gln309 were consistently involved in stabilizing inhibitor molecules within the binding pocket of CYP51-related fungal proteins. Their study emphasized the significance of hydrophobic residues like Ile197, Phe198, and Ala218 in stabilizing potent inhibitors, while hydrogen bonding with residues like Tyr169 and Gln309

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enhanced binding specificity and structural stability. Our results demonstrate a strikingly similar interaction pattern for Phytol, Oleic acid, and Caryophyllene, underlining their promising potential as antifungal agents targeting the CYP51 system. Comparatively, Ketoconazole was found to form multiple hydrogen bonds (e.g., with Asp173, Glu194, Arg210, Asp214, Ser222), reflecting a more hydrogen-bond-dominant mode of binding, consistent with its known mechanism of targeting ergosterol biosynthesis and secondary fungal targets like CYP51. Thus, the multi-modal binding interactions exhibited by natural compounds, particularly Phytol, offer promising alternatives for overcoming fungal resistance, possibly through a dual inhibition mechanism involving membrane disruption and CYP51 inhibition.

Insights into Molecular Interactions and Mechanistic Implications

The molecular docking analysis revealed that Phytol primarily stabilizes within the gyrB active site through extensive hydrophobic interactions with key residues crucial for ATP binding and catalytic activity. Notably, residues such as Val93, Ile94, and Val97 in *E. coli* gyrB form essential hydrophobic contacts, supporting the notion that Phytol can disrupt the enzymatic machinery critical for DNA supercoiling.[28] By targeting these conserved residues, Phytol likely interferes with the coupling of ATP hydrolysis to DNA supercoiling, a hallmark function of DNA gyrase, ultimately leading to bacterial growth inhibition. In *C. albicans*, Phytol demonstrated similar hydrophobic interactions with key residues of CYP51, suggesting a potential dual action against both bacterial gyrB and fungal CYP51, a key enzyme in ergosterol biosynthesis. This dual inhibition could disrupt both DNA replication and membrane integrity, providing a powerful mechanism against fungal growth. Oleic acid displayed binding features indicative of an amphipathic mechanism, with moderate hydrophobic and polar interactions. Its amphiphilic nature suggests a dual mechanism: membrane disruption combined with gyrB inhibition, potentially enhancing bacterial membrane permeability and facilitating intracellular entry to exert inhibitory effects. Similarly, in *C. albicans*, Oleic acid's interactions with CYP51 may contribute to membrane destabilization, complementing its inhibitory effect on gyrB and offering a broader spectrum of antimicrobial action. Such multi-faceted action aligns with previous findings on antimicrobial fatty acids.[29] Pyridine-3-carboxamide, known for interfering with Nicotinamide Adenine Dinucleotide (NAD^+) metabolism, engaged active-site residues such as Asn46 and Val120 in gyrB, indicating its potential to act as a multi-target antimicrobial agent by inhibiting enzymatic pathways beyond DNA gyrase.[30] In CYP51, its ability to form hydrogen bonds with residues such as His373 and Tyr505 further highlights its broad-spectrum inhibitory effects, potentially impacting fungal sterol synthesis in addition to bacterial DNA supercoiling. This broader impact on microbial metabolism could significantly reduce bacterial and fungal adaptability and survival under stress. Caryophyllene, a well-documented sesquiterpene with antimicrobial activity, demonstrated notable interactions with hydrophobic residues in both gyrB and CYP51. It was suggested to inhibit not only gyrB but also impact quorum sensing and biofilm formation mechanisms in bacteria, weakening bacterial virulence and persistence. Additionally, its interaction with fungal CYP51 may help mitigate resistance mechanisms in *C. albicans*, making it a promising candidate for tackling persistent and resistant infections. Its ability to modulate biofilm dynamics could have profound implications for treating chronic infections caused by both bacteria and fungi. Together, these insights underscore the broad-spectrum antimicrobial potential of natural gyrB (gyrB gene encodes the B subunit of DNA gyrase) and CYP51 inhibitors (It belongs to the cytochrome P450 monooxygenase is a fungal-specific sterol), functioning through multi-pronged mechanisms including enzymatic inhibition, membrane disruption, and interference with microbial communication systems. This dual targeting of bacterial and fungal systems presents a promising strategy for overcoming multidrug-resistant infections.

Potential advantages over conventional antibiotics

Unlike standard antibiotics that typically target bacterial cell walls or protein translation machinery, the identified natural compounds offer a multi-pronged approach, interfering with membrane integrity, cellular energy balance, and DNA replication. Such mechanisms could reduce the likelihood of rapid resistance development.[31–33] Moreover, the natural origin of these compounds may support lower toxicity profiles and better biocompatibility, key features for future therapeutic development[34-36] The findings of this study suggest that naturally derived molecules such as Phytol, Oleic acid, Caryophyllene, and Pyridine-3-carboxamide possess strong binding affinities to critical microbial targets and could serve as lead candidates for the development of next-generation antimicrobials.[37,38] Further *in vitro* and *in vivo* validation studies are necessary to confirm their efficacy, assess their pharmacokinetics, and determine potential synergistic effects with existing antimicrobial agents.

CONCLUSION

This study demonstrates that natural compounds, particularly Phytol, exhibit significant binding affinity and favorable interactions with the gyrB subunit of both bacterial and fungal pathogens, including CYP51 in *C. albicans*. Through key hydrophobic contacts and hydrogen bonding within the active sites of both gyrB and CYP51, these compounds potentially disrupt the essential DNA supercoiling function of gyrB and the ergosterol biosynthesis pathway in fungi, thereby impairing microbial replication, survival, and membrane

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integrity. Comparative analysis with previous literature validates the observed interaction profiles and emphasizes the therapeutic promise of targeting both gyrB and CYP51 for antimicrobial and antifungal development. Phytol, due to its strong and consistent binding across diverse organisms, emerges as a particularly potent lead compound for both bacterial and fungal pathogens. These insights provide a foundation for further experimental validation and structure-based optimization of natural gyrB and CYP51 inhibitors as next-generation antimicrobial and antifungal agents.

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ABBREVIATIONS

ATP: Adenosine Triphosphate; **GC-MS:** Gas chromatography-mass spectrometry; **MDR:** Multidrug-resistant; **NAD:** Nicotinamide Adenine Dinucleotide; **PDB:** Protein Data Bank; **PDBQT:** Protein Data Bank, Partial Charge (Q) and AutoDock atom type (T); **SDF:** Structure Data File; **UniProt:** Universal Protein Resource; **WHO:** World Health Organization.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

The authors declare that no funds, grants, or other supports were received during the preparation of this manuscript.

AUTHORS CONTRIBUTION

All the authors contributed to the conception, investigation, and design, Writing - original draft preparation of the study. Material preparation, data collection and analysis were performed by **Subana Suyambumani, Jeevan Pandiyan, Gnanendra Shanmugam**. The manuscript was reviewed, Methodology, Formal analysis and Writing - original draft preparation, Writing - review and editing, Resources by **Maghimaa**.

DATA AVAILABILITY STATEMENT

The dataset analysed during the current study are available from the corresponding author, upon reasonable request.

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