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RESEARCH ARTICLE

Pharmacological Evaluation of Bakuchiol From *Psoralea corylifolia* L. as Potent Antimicrobial Agent Against *Staphylococcus aureus*

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ABSTRACT

The surge in multidrug resistance in *Staphylococcus aureus* is the pressing need to identify novel alternatives to combat antimicrobial resistance effectively. Bakuchiol is a bioactive prenylated phenolic meroterpene largely abundant in the seeds of *Psoralea corylifolia*. In this study, we present the biological assessment of bakuchiol derived from *P. corylifolia* as an antimicrobial agent. *S. aureus*, a significant opportunistic pathogen, attracts global concern for its biofilm formation and resilience against numerous antibiotics, escaping antibiotic pressure. The primary screening of bakuchiol as an antimicrobial agent against *S. aureus* delineated its potential as a strong bactericidal agent with a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 2 and 8 μg/mL, respectively. Importantly, bakuchiol also exhibited low toxicity against HepG2 cells, showing a favorable selectivity index (SI) of 14.4. Furthermore, bakuchiol demonstrated comparable activity (MIC = 2 μg/mL) against a laboratory-generated ciprofloxacin-resistant mutant of *S. aureus*. Bakuchiol could significantly inhibit the biofilm formation of *S. aureus* in a dose-dependent manner with minimum biofilm inhibitory concentration $(MBIC)_{50}$ of 0.956 μg/mL. Bakuchiol effectively inhibited DNA gyrase supercoiling activity at a concentration eight times the MIC, establishing DNA gyrase inhibition as the mechanism of action for bakuchiol. Our findings suggest bakuchiol as a potential therapeutic agent for *S. aureus*-mediated nosocomial infections.

1 | Introduction

Psoralea corylifolia L. (syn. *Cullen corylifolium* L. Medik), commonly known as Bakuchi, belongs to the Fabaceae/Leguminosae family and is extensively utilized in traditional Indian and Chinese medicinal systems. This plant contains various phytoconstituents, including bakuchiol, psoralen, psoralidin, isopsoralen, and bavachin [1]. The seeds have the highest medicinal value as they harbor the most bioactive phytoconstituents like sterols, flavonoids, chalcones, psoralens, terpenes, and, notably, the meroterpene bakuchiol. Bakuchiol, the prenylated phenolic meroterpene, was first extracted from *P. corylifolia* in 1966 [2, 3]. Bakuchiol, also known as Chiba, is the most abundant compound in *P. corylifolia* seeds, constituting 6.24% of the dried seed weight [3]. The presence of "4-hydroxystyryl moiety" in bakuchiol is suggested to have a role in its bioactivity [1]. Despite *P. corylifolia* being the primary plant source for larger-scale bakuchiol extraction, other species like *Fructus psoraleae*,

Psoralidium tenuiflorum, *Prosopis glandulosa*, *Ulmus davidiana*, *Otholobium pubescens*, and *Piper longum* also contain bakuchiol [3].

Extensive research has highlighted bakuchiol's diverse pharmacological activities, such as anti-inflammatory, antibacterial, anticancer, antioxidant, antidepressant, immunosuppressive, hypoglycemic, estrogenic activities, and protection against liver microsome oxidation. It has shown promising protective effects on organs such as the heart and liver and has implications in various diseases and conditions like liver fibrosis, myocardial ischemia-reperfusion injury, osteoporosis, anxiety, neurodegenerative diseases, and human carboxylesterase 2 (hCE2) inhibition [1–4]. The recent interest in bakuchiol has implicated its applications in dermatological conditions like acne, psoriasis, and aging. The dermatological applications of bakuchiol have been attributed to its structural similarity to retinol (vitamin A), which has led to its potential bioactivity mimicry [2].

Studies by Katsura et al. have demonstrated bakuchiol's antibacterial activity against oral microorganisms, inhibiting the growth of *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus sanguis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Porphyromonas gingivalis*. Bakuchiol exhibits bactericidal effects and is effective against adherent *S. mutans* cells, suggesting its potential use as a food additive and in mouthwash to prevent and treat dental caries [5]. Additionally, bakuchiol acts as an antibacterial compound by inhibiting nucleic acid synthesis, leading to bacterial death via DNA spiral degradation and topoisomerase II enzyme inhibition. Bakuchiol has also been shown to inhibit methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, mycobacteria, and dermatophytes [4, 6, 7].

The present study aims to determine the antimicrobial potential of bakuchiol from *P. corylifolia* against a multidrug-resistant strain of *S. aureus*. The objective of the study was achieved by conducting detailed in vitro studies that validated the antimicrobial properties and elucidated the mechanism of action for bakuchiol.

2 | Materials and Methods

2.1 | General Experimental Procedures

The chemicals were obtained from Sigma-Aldrich Company and used as received. The bulk solvents were used only after distillation. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-Avance III HD 500 MHz NMR spectrometer using internal standard tetramethylsilane (TMS). The TMS was referenced to the residual proton/carbon in the NMR solvent ($CDCl_3$, 7.26/77.1 ppm; CD_3OD, 3.31/49.0 ppm, and DMSO-d_6 2.50/39.5 ppm). The chromatographic purifications were performed using silica gel (#60–120 or #100–200 from E. Merck, Germany). The thin-layer chromatography (TLC) was performed on pre-coated silica gel 60 GF254 aluminum sheets from Merck (Germany). The spots of compounds were visualized under short-wave (254 nm) ultraviolet light. HRMS spectra of the compounds were recorded on HRMS-6540-UHD machines.

2.2 | Plant Material

The seeds of *P. corylifolia* were purchased from the local market in Varanasi (January 2020 by Nancy Tripathi, Research Scholar, Department of Pharmaceutical Engineering & Technology, IIT (BHU)). The seeds were authenticated by Prof. N. K. Dubey, Department of Botany, Banaras Hindu University, Varanasi, India. The specimen sample of *P. corylifolia* seeds was also preserved (Voucher specimen no. Faba.2023/01) in the Department of Botany, Banaras Hindu University, Varanasi, India.

2.3 | Extraction and Isolation of Bakuchiol

The seeds (500 g) were coarsely grounded and extracted via cold maceration with chloroform:methanol mixture in a 1:1 (v/v) ratio. The extraction cycle was repeated three times, and finally, the extract was dried under reduced pressure to get a viscous crude extract. The extract (ethyl acetate fraction) was then dissolved in the minimum amount of solvent and adsorbed over silica gel to make the slurry. The slurry was loaded over the silica-gel column chromatography to extract bakuchiol. The columns were run using ethyl acetate in hexane, starting from 100% hexane to 100% ethyl acetate. The fractions obtained were repeatedly purified using silica gel column chromatography to get a pure compound. The obtained compound was characterized via NMR spectroscopy and mass spectrometry, and the structures were confirmed by comparing the NMR data with the reported literature (Figure S1–S4).

Bakuchiol. $C_{18}H_{24}O$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, $J = 8.2$ Hz, 2*H*), 6.81 (d, $J = 8.4$ Hz, 2*H*), 6.28 (d, $J = 16.2$ Hz, 1*H*), 6.09 (d, $J = 17.7$ Hz, 1*H*), 5.92 (dd, $J = 19.6$, 10.8 Hz, 1*H*), 5.20–5.11 (m, 1*H*), 5.10–5.00 (m, 2*H*), 1.99 (d, $J = 8.9$ Hz, 2*H*), 1.71 (s, 3*H*), 1.62 (s, 3*H*), 1.57–1.49 (m, 2*H*), 1.23 (s, 3*H*). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.7, 146.0, 135.8, 131.3, 130.8, 127.4, 126.5, 124.8, 115.4, 111.9, 42.5, 41.3, 25.7, 23.4, 23.3, 17.7. HRMS *m/z*: $[M+H]^+$ calc. 257.1900, obs. 257.2188 [8].

2.4 | Molecular Docking Studies

Molecular docking studies were performed using the AutoDock 4.2 software. The structure coordinates of DNA gyrase in complex with ciprofloxacin (PDB: 2XCT) were obtained from the RCSB. To ensure accurate results, the pdb2pqr web server was utilized to assign the appropriate protonation state to the residues (https://server.poissonboltzmann.org/pdb2pqr). During the process, all water molecules, ligands, and ions were removed, whereas nonpolar hydrogen atoms were eliminated. Gasteiger charges were added using M.G.L Tools 1.5.7.rc1. AutoDock utilized Autogrid4 to compute maps. The docking study employed the Lamarckian genetic algorithm (LGA) with specific parameters: 100 runs, 150 population sizes, 27 000 generations, and 2 500 000 energy evaluations. A "semiempirical free-energy force field" was employed to evaluate the conformations during the docking simulation. The visual analysis of the docked pose and the molecular interactions was carried out using Desmond Maestro (Figure S6). Subsequently, the best conformation with the most favorable binding energy within the largest cluster at 2.0 Å was

selected after the successful completion of the docking simulation [9].

2.5 | Molecular Dynamics Simulation

MD simulation was used to study the dynamic behavior of protein–ligand complexes. A 250 ns simulation study was performed to evaluate the stability of the protein–ligand complex of identified hits using GROMACS 2020 software [10]. The best docked pose of the ligand was employed to create ligand topology files using the CHARMM-GUI web server's input generator for GROMACS, utilizing the CHARMM-36 M force field [11].

The protein was solvated using the TIP3P water model and neutralized by adding Na^+ and Cl^- ions through the Monte Carlo ion placement method. The system's energy was minimized over 50 000 steps using the steepest descent algorithm, followed by equilibration for 1 ns in the NVT ensemble with restrained protein. The temperature was maintained at 303.15 K with a Nosé–Hoover thermostat, applying a 1.0 ps coupling constant to both protein and nonprotein atoms [12]. Subsequently, the system was equilibrated for 1 ns in the NPT ensemble at 1 bar pressure using a Parrinello–Rahman barostat with a 1 ps coupling constant. The system then underwent a 150 ns isobaric-isothermal MD production simulation with a 2 fs time step throughout. The LINCS algorithm was used to constrain bond lengths, whereas long-range electrostatic interactions were handled using the particle mesh Ewald (PME) method with a 1.2 nm cut-off radius and 0.16 nm grid spacing [13, 14]. The van der Waals interactions were cut off at 1.2 nm. Post-simulation, the trajectories were analyzed for root mean square deviation (RMSD), root mean square fluctuations (RMSF), radius of gyration (rGy), hydrogen bond analysis, and solvent accessible surface area (SASA), and the results were plotted.

2.6 | Evaluation of the Antimicrobial Activity of Bakuchiol

2.6.1 | Bacterial Strains and Growth Conditions

The experiments were performed with a set of different bacteria: *Acinetobacter baumannii* (ATCC 19606), *S. aureus* (ATCC 29213), and *Pseudomonas aeruginosa* PAO1 (ATCC 15692). Strains were grown on Mueller–Hinton agar (MHA). For growth in liquid culture, single colonies are picked from the agar plates, inoculated in cation-adjusted Mueller–Hinton broth (MHB), and incubated at 37°C.

2.6.2 | Generation of Resistant Mutants of Ciprofloxacin In Vitro

A bacterial suspension of 10^9 CFU was prepared with *S. aureus*, and 100 µL of it was plated on MHA plates containing ciprofloxacin concentrations equivalent to 8× and 16× minimum inhibitory concentration (MIC) values. Plates were further incubated for more than 72 h. Colonies that appeared after 72 h were isolated and grown in liquid broth for susceptibility against ciprofloxacin. Colonies that showed ≥8-fold change in MIC of ciprofloxacin were taken up for cross-resistance studies against bakuchiol [12].

2.6.3 | Susceptibility Testing of Extract and Bakuchiol Against SAP (*S. aureus*, *A. baumannii*, and *P. aeruginosa*) Pathogens

Susceptibility testing was carried out on the extract and isolated bakuchiol by determination of MIC using the broth microdilution method. MIC is defined as the lowest concentration of compound that inhibits visible bacterial growth. Extract and bakuchiol were started from 128 µg/mL and then serially diluted in a 96-well round bottom plate along with ciprofloxacin as reference standard. Bacterial cultures were prepared in MHB from a previously streaked MHA plate. The optical density (OD600) of the inoculum was adjusted to 0.08, followed by 1:100 dilution for the final inoculum of 10^6 CFU/mL, which was added to all the wells. Further incubation of plates was done for 18 h at 37°C, followed by observations of visible growth to note MIC values [15].

2.6.4 | Evaluation of Minimum Bactericidal Concentration (MBC)

S. aureus (ATCC 29213) culture was adjusted to OD_{600} of 0.08 and further dilution of 1:100 to obtain the inoculum. Inoculum was aliquoted into a 24-well plate along with different concentrations of bakuchiol, and the plates were kept in incubation at 37°C for 6 h. Bacterial viability was determined by calculating the CFU/mL on agar plates [16].

2.6.5 | Time-Kill Kinetics Study

Time-kill kinetics studies were performed with different concentrations of bakuchiol against *S. aureus* (ATCC 29213). Bakuchiol at its 2 µg/mL (MIC), 8 µg/mL (4× MIC), 16 µg/mL (8× MIC), and ciprofloxacin at 0.125 µg/mL (MIC) as reference standard in triplicates was evaluated using a time-kill curve method. Bacterial suspension was prepared using an adjusted OD_{600} of 0.08 and further 1:100 dilution for around 10^6 CFU/mL. A volume of 450 µL of final bacterial inoculum was incubated with 50 µL bakuchiol at different concentrations. CFU/mL was determined by serial dilution method on MHA plates at 0, 3, 6, 9, and 12 h of incubation at 37°C. Log_{10}CFU/mL was plotted against time to obtain the kill curve [17].

2.6.6 | Minimum Biofilm Inhibitory Concentration (MBIC) Determination

The inhibitory activity of bakuchiol against biofilm formation by *S. aureus* ATCC 29213 was determined in a 96-well flat-bottom plate. Culturing of bacteria was done overnight in tryptone soya broth with 2% glucose supplementation (TSBG), and OD_{570} was adjusted to 0.015 after 1:100 dilution. For performing the assay, bakuchiol was added in different concentrations by two-fold serial dilution along with the culture and incubated at 37°C for 48 h. Further, media was discarded, and wells were

washed thrice with sterile PBS to remove the planktonic cells; further methanol was added for fixation and air dried. Staining of biofilms was done using 0.1% (w/v) crystal violet and washed with sterile water until negative control (without inoculum) became colorless. Quantification of biofilms was done using 200 µL of 30% acetic acid, and absorbance was measured at 595 nm using BioTek Cytation 5 multimode reader. $MBIC_{50}$ was calculated using GraphPad Prism 8 software and is defined as the concentration at which 50% inhibition of biofilms is observed [18].

2.6.7 | DNA Gyrase Inhibitory Assay

The *Escherichia coli* DH5α strain containing pUC19 plasmid was grown overnight in LB broth supplemented with 50 µg/mL ampicillin. Cells were pelleted down at 4000 rpm for 10 min and resuspended in fresh LB broth to adjust the OD_{600} to 2.0. The bacterial suspension was then subjected to treatment with DMSO; bakuchiol at 2 µg/mL (MIC), 8 µg/mL (4× MIC), 16 µg/mL (8× MIC), and 32 µg/mL (16× MIC); and ciprofloxacin (0.125 µg/mL) and incubated at 37°C with 200 rpm shaking. Following the incubation, cells were harvested, and isolation of pUC19 was done with a QIAprep spin miniprep kit (Qiagen). The topology of the plasmid DNA was evaluated using 0.8% agarose gel electrophoresis [19, 20].

2.6.8 | Cell Cytotoxicity

Bakuchiol was screened for cytotoxicity against HepG2 cells (ATCC HB-8065) using an MTT assay. 10^5 cells/well were seeded in a 96-well plate and incubated at 37°C with 5% CO_2 for 24 h. Bakuchiol was treated in twofold serial dilution with concentrations ranging from 128 to 0.06 µg/mL for 24 h. Following incubation, MTT was added at 2.5 mg/mL to the wells and incubated for another 4 h. Residual media was decanted, and DMSO was added to solubilize the formazan crystals. Absorbance was measured at 570 nm for evaluation of CC_{50}. CC_{50} is defined as the concentration at which there is a 50% reduction in cell viability [2].

3 | Results

3.1 | Isolation, Purification, and Characterization of Bakuchiol

The seeds of *P. corylifolia* were coarsely grounded and extracted with a mixture of chloroform:methanol (ratio 1:1) via cold maceration with intermittent stirring. The cycle was repeated three times to ensure complete extraction. Both the extracts were dried, and the resultant extracts were weighed to get the final yield of approximately 113 g (22.6%). The extract was subjected to column chromatography for the isolation of bakuchiol using *n*-hexane:ethyl acetate as mobile phase in a step gradient manner starting from 100% hexane to 100% ethyl acetate. Different fractions were collected at each increasing polarity and were pooled on the basis of the TLC profiling. The obtained fraction (5% ethyl acetate in hexane) was subjected to repeated column chromatography to obtain a pure compound, which resulted in the isolation of bakuchiol (Figure 1). The structure determination of the isolated compounds was done using NMR spectroscopy and mass spectrometry, and the spectra were compared with those of the reported literature [8].

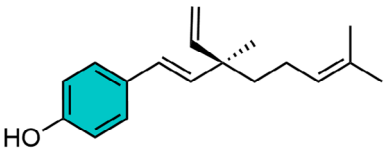

FIGURE 1 | Structure of isolated (+)-bakuchiol.

3.2 | Evaluation of the Antimicrobial Activity of Bakuchiol

Bakuchiol was evaluated for antibiotic susceptibility testing against Gram-positive and Gram-negative bacterial pathogen panel consisting of *A. baumannii* (ATCC 19606), *S. aureus* (ATCC 29213), and *P. aeruginosa* PAO1 (ATCC15692) using ciprofloxacin as a standard drug. The extract and bakuchiol were tested in the concentration range of 128–0.06 µg/mL. The MIC was determined, and the results are listed in Table 1.

Both the extract and isolated bakuchiol exhibited promising and selective inhibitory activity against *S. aureus* at 32 and 2 µg/mL, respectively (Table 1), which further led us to test bakuchiol against a laboratory-generated ciprofloxacin mutant of *S. aureus*, and it was observed that bakuchiol was equally effective against a ciprofloxacin-resistant strain of *S. aureus* (Table 2).

3.2.1 | MBIC Assay

The biofilm inhibitory activity for bakuchiol was evaluated by growing *S. aureus* ATCC 29213 for 48 h with different concentrations of bakuchiol and ciprofloxacin as the reference standard. Biofilm inhibitory activity was assessed using a quantitative assay utilizing crystal violet for staining the biofilms (Figure 2).

TABLE 1 | Minimum inhibitory concentration (MIC) (µg/mL) of extract and bakuchiol against *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

	Acinetobacter baumannii **MIC (µg/mL)**	***Staphylococcus aureus*** **MIC (µg/mL)**	***Pseudomonas aeruginosa*** **MIC (µg/mL)**
Extract	> 128	32	> 128
Bakuchiol	> 128	2	> 128
Ciprofloxacin	0.25	0.25	0.25

TABLE 2 | Minimum inhibitory concentration (MIC) (μg/mL) of bakuchiol against ciprofloxacin-resistant *Staphylococcus aureus* mutant.

Compound	*Staphylococcus aureus* 29213 Cip^r MIC (μg/mL)
Bakuchiol	2
Ciprofloxacin	2

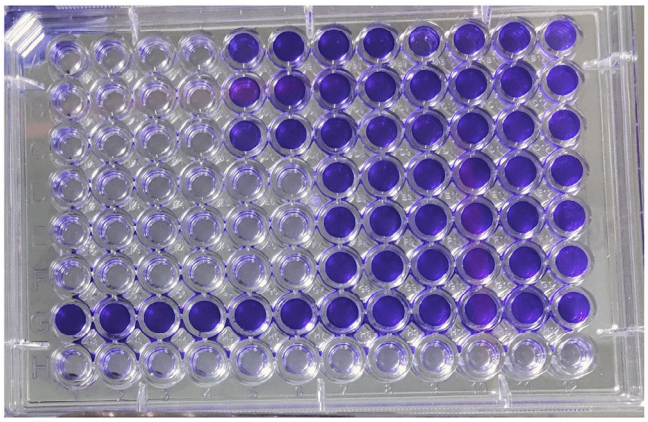

FIGURE 2 | Biofilm formation after crystal violet staining. The experiment was performed with three technical replicates and repeated at least once (n = 3).

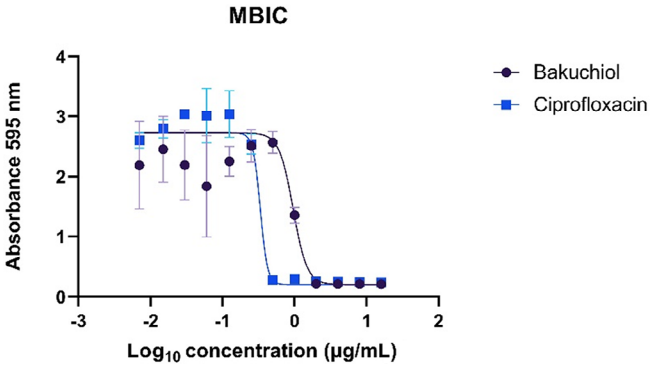

FIGURE 3 | MBIC graph of bakuchiol. Data are expressed as mean ± SD of triplicate for each condition of the experiment (n = 3).

Bakuchiol showed concentration-dependent inhibition of the biofilms (Figure 2) with $MBIC_{50}$ of 0.956 ± 0.02 μg/mL and ciprofloxacin, which was used as the control drug (positive control) which showed $MBIC_{50}$ value of 0.335 ± 0.01 μg/mL (Figure 3). The blank media was used as negative control.

3.2.2 | MBC Assay

The bacteria were exposed for 6 h at different concentrations of bakuchiol in multiples of MIC ranging from 1× MIC to 8× MIC. It was observed that bakuchiol shows a 3 Log_{10} reduction in the viable bacterial load, which confirms that bakuchiol is bactericidal (99.9% kill) at 4× and 8× MIC concentrations (Figure 4).

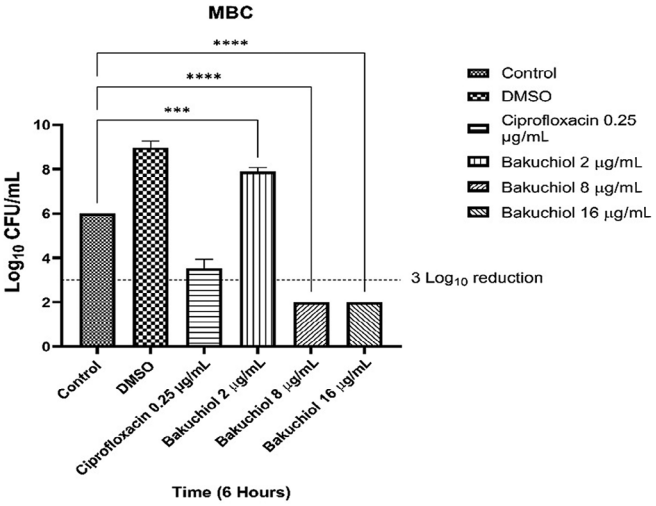

FIGURE 4 | MBC graph of bakuchiol at different concentrations. Statistical difference ***$p < 0.001$, one-way ANOVA in CFU between bacteria treated with 2× MIC of bakuchiol and initial inoculum count at the start of the experiment, ****$p < 0.0001$, one-way ANOVA in CFU between bacteria treated with 4×, 8× MIC and initial inoculum. Data are expressed as mean ± SD of triplicate for each condition of the experiment ($n = 3$). MBIC, minimum biofilm inhibitory concentration.

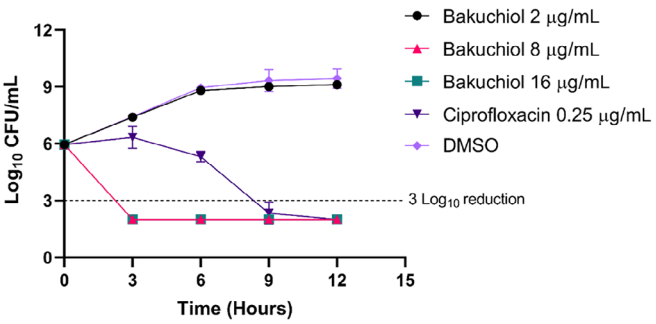

FIGURE 5 | Time-Kill analysis of bakuchiol. Statistical difference ****$p < 0.0001$, one-way ANOVA in CFU between bacteria treated with 4× and 8× MIC of bakuchiol and initial inoculum count at the start of the experiment. Each timepoint represents the mean Log_{10} ± SD of three readings of CFU ($n = 3$).

3.2.3 | Time-Kill Kinetics Assay

Bakuchiol was further evaluated for time- and dose-dependent killing using a time-kill curve assay. It was observed that bakuchiol was bactericidal at 4× and 8× MIC concentrations in just 3 h of incubation, and there was no emergence of resistance even after 12 h of exposure (Figure 5).

3.2.4 | Cytotoxicity Assay Against HepG2 Cells

Bakuchiol was evaluated for its cytotoxicity against HepG2 cells using an MTT assay. Tamoxifen was used as a reference standard. The cytotoxicity results were expressed as CC_{50} (lowest concentration of a compound that causes the death of 50% of viable cells), and selectivity index (SI, ratio between CC_{50} and MIC) values are given in Table 3. Bakuchiol displayed a favorable

TABLE 3 | Cytotoxicity profile of bakuchiol against HepG2 cells.

	Bakuchiol
CC_{50} (μg/mL)	28.80 ± 0.43
SI	14.4

Abbreviation: SI, selectivity index.

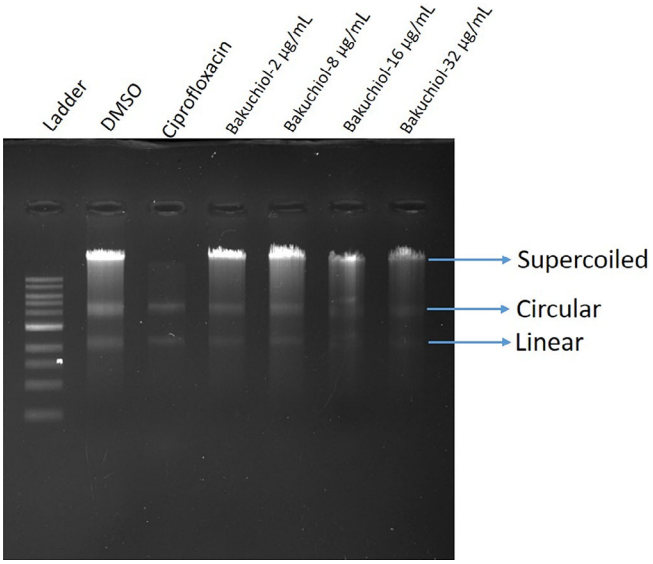

FIGURE 6 | DNA supercoiling assay of bakuchiol. Refer to Figure S5 for full-size gel image.

SI, which was greater than 10, indicating the safety as well as antimicrobial efficacy of bakuchiol. Data are expressed as mean ± SD of triplicate for each condition of experiment ($n = 3$).

3.2.5 | DNA Supercoiling Assay

The target validation of bakuchiol was done using a DNA supercoiling assay. A decrease in the band intensity of the plasmid was observed, along with an increase in the bakuchiol concentration, confirming its role as a potential gyrase inhibitor targeting supercoiling in the plasmid (Figure 6).

3.3 | Molecular Docking

Further, the molecular docking study of bakuchiol was performed with *S. aureus* DNA gyrase protein (PDB: 2XCT) to study its possible binding modes and interactions with the protein. The binding energy of bakuchiol was compared against the ciprofloxacin standard. Bakuchiol displayed good binding energy for the *S. aureus* DNA gyrase protein which was comparable to ciprofloxacin (Table 4). The 2D interaction diagrams of bakuchiol and ciprofloxacin with *S. aureus* gyrase are provided in Figure S5. The docking results were shown to support the inhibition of *S. aureus* gyrase protein by bakuchiol which was similar to that of ciprofloxacin.

4 | Molecular Dynamics Simulation

The RMSD values of C-alpha atoms in the docked complexes were analyzed to evaluate structural variations and stability for each complex. Initially, the RMSD profiles of the ciprofloxacin–DNA gyrase and bakuchiol–DNA gyrase complexes were similar, maintaining stability and showing minimal RMSD fluctuations. Throughout the simulation, RMSD values for each complex ranged between 0.0005 and 1.4 nm, indicating structural stability. The average RMSD values for the ciprofloxacin and bakuchiol complexes were 0.99 and 0.73 nm, respectively (Figure 7A). Surface area changes for the ligand–protein complexes were also assessed through SASA values (Figure 7B). Early in the simulation, both complexes demonstrated stability, and after 70 ns, their SASA values stabilized, indicating a reduction in protein surface area. The rGy, an indicator of complex mobility, showed higher values at the beginning and end of the simulation, suggesting a labile nature of the protein. The bakuchiol complex displayed more fluctuation than the ciprofloxacin complex, implying greater conformational rigidity and stability in the ciprofloxacin complex (Figure 7C). Hydrogen bonding, crucial for system stability, was similar in both complexes throughout the simulation (Figure 7D). RMSF analysis showed no significant differences between the ciprofloxacin and bakuchiol complexes bound to DNA gyrase (Figure S7), with RMSF values not exceeding 0.5 Å, suggesting a compact protein structure due to minimal residue movement. Although both complexes showed stable RMSF patterns, closer inspection revealed reduced residue movement in the bakuchiol complex compared to the ciprofloxacin complex, indicating that bakuchiol binding resulted in a more stable drug-protein complex (Figure S7).

5 | Discussion

Crude extracts and phytoconstituents from various parts of *P. corylifolia* are known to show a wide range of therapeutic activities ranging from antimicrobial, anticancer, anti-inflammatory, and anti-oxidative properties. *S. aureus* is a major pathogenic strain causing various soft tissues and skin disorders in humans. Due to evolving resistance mechanisms, treating infections caused by *S.* aureus has been difficult. In this study, we extracted bakuchiol from *P. corylifolia* and evaluated its activity against *S. aureus*, *P. aeruginosa*, and *A. baumannii*. Bakuchiol showed strong antimicrobial activity against the Gram-positive bacteria *S. aureus* (MIC—2 μg/mL) but lacked significant antimicrobial activity against Gram-negative strains, which led us to further evaluate its activity against laboratory-generated ciprofloxacin-resistant *S. aureus* strain. Bakuchiol was found to be equally potent against *S. aureus* Cip^R strain where it inhibited bacterial growth at 2 μg/mL. The ability to produce biofilms by *S. aureus* is the main reason behind the relapse of infection, and the heterogeneous population of persisters present within the biofilm are known to tolerate antibiotics, further contributing to treatment failures. Our study showed dramatic inhibition of biofilm formation with an MBIC of 0.956 μg/mL, suggesting that bakuchiol can target even the persistent populations that are difficult to treat otherwise. Previous studies that reported the antimicrobial properties of bakuchiol omitted the safety evaluation of the drug. In this study, we also reported the cell cytotoxicity of bakuchiol against the HepG2 cell line; a CC_{50} of

TABLE 4 | Docking score and ligand interaction of bakuchiol with *Staphylococcus aureus* gyrase.

Ligands	Binding energy (kcal/mol)	Ligand efficiency (kcal/mol)	Ligand Interactions with *Staphylococcus aureus* gyrase (PDB: 2XCT)
Bakuchiol	−6.860	−0.361	D:Phe 1097 (Pi–Pi stacking, hydrophobic), D:Asp 1116 (charged), D:Ala 1118 (hydrophobic), D:Ile 1264 (hydrophobic)
Ciprofloxacin	−7.120	−0.297	D:Pro 1080 (hydrophobic), D:His 1081 (Polar), D:Tyr 1150 (hydrophobic), D:Asp 1151 (charged)

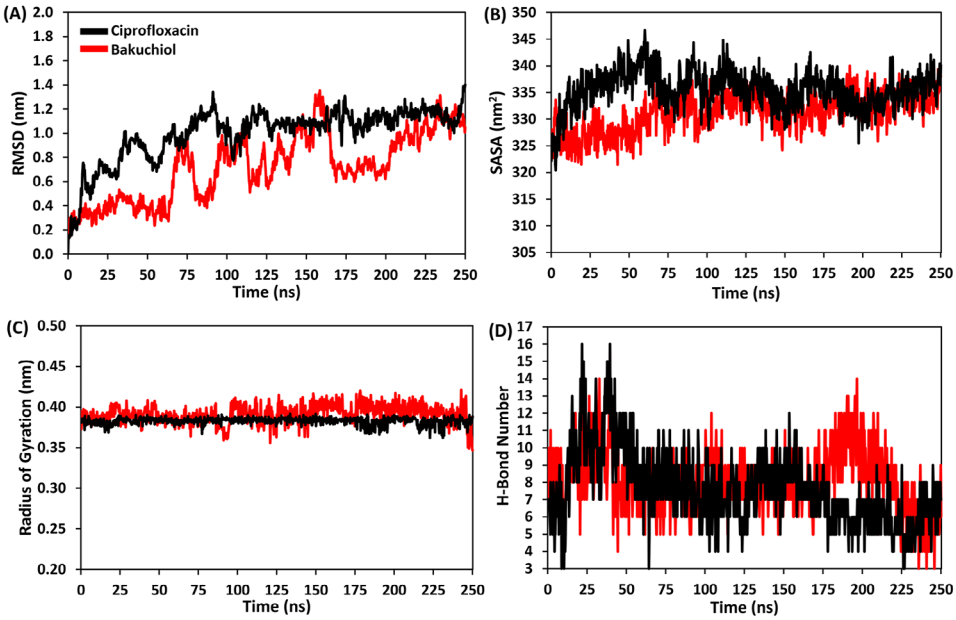

FIGURE 7 | Molecular dynamics simulation of ciprofloxacin- and bakuchiol-bound to DNA gyrase where (A) root mean square deviation of the C-alpha atoms, (B) solvent accessible surface area, (C) radius of gyration, and (D) number of hydrogen bonds.

28.80 μg/mL was observed. We further calculated the SI, which is a ratio of CC_{50} and MIC value, and an index higher than 10 is safe for further use. SI value of bakuchiol is 14.4, which confirms that it can be considered for further studies.

DNA gyrase is an attractive target for developing novel antimicrobial drugs. Previous studies have demonstrated that topological evaluation of pUC19 plasmid in an agarose gel can suggest the inhibition of DNA supercoiling. Similarly, in this study, we used the DNA gyrase inhibitory assay to establish the mechanism of action for bakuchiol for the first time. Bakuchiol showed a concentration-dependent reduction in the supercoiled state of DNA, which confirms that bakuchiol inhibits the DNA gyrase enzyme. Further studies with various clinical isolates of *S. aureus* in future could give us an idea about the killing efficacy across different drug-resistant strains for bakuchiol.

6 | Conclusion

In summary, bakuchiol was extracted and isolated from *P. corylifolia* and evaluated for its antimicrobial efficacy against the *S. aureus* strain. With an MIC of 2 μg/mL, bakuchiol demonstrated strong effectiveness against *S. aureus* while maintaining non-toxicity to HepG2 cells, yielding a favorable SI of 14.4. The compound also displayed comparable activity against a laboratory-generated ciprofloxacin-resistant mutant of *S. aureus*. Additionally, bakuchiol manifested bactericidal effects and inhibited DNA supercoiling in a DNA gyrase inhibition assay. These findings suggest that bakuchiol shows promise for further exploration as a potential antimicrobial agent. Our data indicate that bakuchiol can act as a parent molecule for generating more potent structural analogs that can be effective against various drug-resistant clinical isolates of *S. aureus* and equally potent in vivo.

Author Contributions

N.T. was involved in the extraction, isolation, and purification of bakuchiol from the extract, in performing in silico studies and writing the original draft. A.R. and P.K.A. did the antimicrobial screening under the supervision of N.P.K. N.B. was involved in structure elucidation. S.K. was involved in the collection of plant material. S.K.J. performed compound characterization, overall supervision, and final review.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are included in the manuscript and associated Supporting Information.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.