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INVESTIGATION OF THE ANTIBACTERIAL POTENTIAL OF (R)-(+) LIMONENE AGAINST FOOD-ASSOCIATED STRAINS OF *PSEUDOMONAS AERUGINOSA* THROUGH IN VITRO AND IN SILICO ANALYSES

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ABSTRACT

Antimicrobial resistance is one of the major global health challenges. Consequently, the effectiveness of conventional antimicrobials has progressively declined due to the emergence of multidrug-resistant strains, making the search for antimicrobial alternatives essential. This study aimed to evaluate the antimicrobial activity of (R)-(+)-limonene against *Pseudomonas aeruginosa* strains isolated from food and to investigate its possible mechanism of action via molecular docking with DNA gyrase B. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays were performed using microdilution in 96-well plates, while the Minimum Inhibitory Concentration for Adhesion (MICA) was assessed in test tubes. In addition, interactions with antimicrobials were investigated using the disk diffusion method, and molecular docking was performed with AutoDock 4.2. The results showed an MIC90 of 1000 µg/mL and an MBC at ratios of 1:1 and 2:1, indicating a bactericidal effect. Regarding anti-adhesive activity, (R)-(+)-limonene inhibited biofilm formation at a dilution ratio of up to 1:8. With regard to combination with antimicrobials, a synergistic effect was observed with ampicillin, gentamicin, tetracycline, ciprofloxacin, ceftazidime, and cefazolin. Additionally, interactions with the active site of the DNA gyrase B enzyme were identified, primarily via hydrophobic, van der Waals, and alkyl/pi-alkyl interactions. These findings reinforce the potential of (R)-(+)-limonene as a promising agent for microbial control and highlight the importance of bioactive compounds derived from essential oils as alternatives or adjuvants for developing new therapeutic strategies in veterinary medicine.

***Keywords*:** Bacteria, Phytotherapy, Natural product, Antimicrobial resistance.

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1. INTRODUCTION

Increasing interactions among animals, humans, and ecosystems have heightened the risk of the spread of emerging and re-emerging diseases. In light of this, this complex health threat requires collaborative action across multiple sectors, known as the One Health approach, which is characterized by an integrated and unifying strategy aimed at promoting, balancing, and optimizing human and animal health, food security, and the preservation of ecosystems in a sustainable manner (Erkyihun et al., 2022).

In this context, antimicrobial resistance represents one of the major global health challenges and a clear example of the *One Health* approach, as it simultaneously affects humans, animals, and the environment, and is directly linked to the excessive and inappropriate use of antimicrobials in various sectors, such as human medicine, agriculture, and livestock farming. Furthermore, the inappropriate use of antimicrobials, inadequate infection control, agricultural waste, environmental and food pollutants, and the migration of people and animals infected with resistant bacteria facilitate the spread of resistance (Velazquez-Meza et al., 2022; Okpalaji et al., 2025).

Given this context, *Pseudomonas aeruginosa* stands out as a highly significant pathogenic Gram-negative bacterium, characterized by multiple virulence factors, biofilm formation, and high antimicrobial resistance (Elfadadny et al., 2024). The widespread distribution of this pathogen is linked to its metabolic versatility, rapid

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multiplication, high adaptability, and ability to grow at low temperatures, all of which contribute to its environmental persistence and its potential to cause foodborne infections (Li et al., 2023; Gao et al., 2023).

The effectiveness of conventional antimicrobials has been progressively diminished by the emergence of multidrug-resistant strains, underscoring the need for safe, effective antimicrobial alternatives (Seukep et al., 2023). In this context, natural compounds such as R-(+)-limonene ($C_{10}H_{16}$), the primary isomer found in citrus peel and the most biologically active, stand out for their broad-spectrum bioactivity and low toxicity, exhibiting antimicrobial effects and showing promise for clinical development (Kesbiç et al., 2022; Hoosen et al., 2025).

This study aims to evaluate the antimicrobial activity of (R)-(+)-limonene against *Pseudomonas aeruginosa* strains isolated from food and to investigate its possible mechanism of action via molecular docking with DNA gyrase B.

2. MATERIALS AND METHODS

2.1. Test Substance

The monoterpene (R)-(+)-limonene was obtained from Sigma-Aldrich® (São Paulo, SP). For the pharmacological assays, the substance was first dissolved in dimethyl sulfoxide (DMSO) and then diluted in distilled water, maintaining the final DMSO concentration below 0.1% (v/v).

2.2. Microorganisms

Strains of *Pseudomonas aeruginosa* (*ATCC 9027*, *Pa43*, *Pa44*, *Pa63*, and *Pa68*) were used and maintained on Mueller-Hinton (MH) agar at 4°C. Bacterial inocula were prepared from cultures incubated overnight in MH medium at 35 ± 2 °C and then suspended in sterile saline until a final concentration of approximately 1.5×10^8 colony-forming units per milliliter (CFU/mL) was reached, adjusted by comparing turbidity to the 0.5 standard on the McFarland scale (Bona et al., 2014).

2.3. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the microdilution method in 96-well plates. 100 µL of double-concentrated Mueller-Hinton broth and 100 µL of (R)-(+)-Limonene were added; subsequently, a two-fold serial dilution was performed, yielding concentrations of 1000, 500, 250, 125, 62.5, and 31.2 µL/mL. The MIC determination was conducted with 10 µL of the microorganism in each well, approximately 1.5×10^8 CFU/mL. As controls, the penultimate well was designated for sterility control, containing only 100 µL of broth, while the last well was designated for the growth control, containing 100 µL of double-concentrated Mueller-Hinton broth and the bacterial suspension. The plates were incubated at 35–37°C for 24 hours; after incubation, the results were first read. Next, 20 µL of sodium resazurin solution (SIGMA), previously dissolved in sterile distilled water at a concentration of 0.01% (w/v)—recognized as a colorimetric redox indicator for bacteria—was added, followed by further incubation at 35–37°C. The MIC was defined as the lowest concentration of the compound required to inhibit visible growth of the evaluated microorganism, as confirmed by the absence of a change in the indicator's color. (Palomino et al., 2002; Ostrosky et al., 2008; CLSI 2012; Bona et al., 2014).

2.4. Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of the monoterpene was also determined for the bacterial strains. After reading the MIC, inoculations (10 µL) of up to three dilutions prior to the MIC value were made into Mueller-Hinton broth (100 µL/well) on a sterile microdilution plate, followed by incubation at 35–37°C for 24 hours. After this period, 20 µL of resazurin was added, and a new incubation was performed at the same temperature. The results were read to confirm the lowest concentration capable of achieving total inhibition of bacterial growth, as evidenced by the absence of a change in the indicator dye's color (Ncube et al., 2008; Guerra et al., 2012).

2.5. Minimum Inhibitory Concentration for Adhesion (MICA)

The minimum inhibitory concentration for adhesion (MICA) of the compound was determined in the presence of 5% sucrose, according to the method described by Albuquerque et al. (2010), with modifications, using concentrations ranging from undiluted to a 1:256 dilution. Based on bacterial growth, the bacterial strain was cultured at 35–37°C in Mueller-Hinton broth; subsequently, 0.9 mL of the subculture was distributed into test tubes, and then 0.1 mL of the solution corresponding to the compound's dilutions was added. Incubation was performed at 35–37°C for 24 hours with tubes tilted at a 30° angle. The results were assessed by visual observation of bacterial adhesion to the tube walls after shaking the tube. The assay was performed in duplicate. The same procedure was performed for the positive control, 0.12% chlorhexidine digluconate. The MICA was defined as the lowest concentration of the agent, in the presence of sucrose, capable of preventing bacterial adhesion to the glass tube.

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2.6. Study of the Combination of R-(+)-limonene with Synthetic Antimicrobials

The study of the product's interaction with antimicrobials was conducted using the solid-medium disk diffusion technique, employing filter paper disks impregnated with the following antimicrobials: ampicillin (APM10-10µg), gentamicin (GEN10-10µg), ciprofloxacin (CIP-5µg), ceftriaxone (CRO-30µg), ceftazidime (CAZ-30µg), penicillin (PEN-10µg), amoxicillin (AMC-30µg), amikacin (AMI-30 µg), levofloxacin (LVX-5 µg), norfloxacin (NOR-10µg), cefazolin (CFZ-30 µg), and cephalothin (CFL-30 µg). In smooth sterile Petri dishes containing Muller Hinton agar medium previously inoculated with the bacterial suspension, discs containing antimicrobials were placed, and 20 µL of the MIC of the test product was added; a negative control was also performed, containing only the antimicrobial discs with the bacterial suspension. Subsequently, the plates were incubated at 35–37°C for 24–48 hours, followed by reading. A synergistic effect was considered when the inhibition zone resulting from the combination of the product and the antimicrobial agent increased by 2 mm or more compared to the zone produced by the antimicrobial agent alone. When the diameter of the zone formed by the combination was smaller than that obtained with the antimicrobial agent alone, the effect was classified as antagonistic. In cases where the zone of inhibition remained unchanged compared to the antimicrobial agent used alone, the effect was considered indifferent (Cleeland & Squires, 1991; Oliveira et al., 2006). All experiments were conducted in duplicate.

2.7. Molecular Docking

Rigid molecular docking simulations were performed using the *P. aeruginosa* DNA gyrase B protein (PDB ID: 7PTF), obtained by X-ray diffraction at a resolution of 1. 32 Å and complexed with novobiocin, whose structure was obtained from the Protein Data Bank (PDB) and loaded into PyMol v.3.1.3 software to remove water molecules and other artifacts. The ligand (R)-(+)-limonene was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format and pre-optimized in Avogadro 1.2.0 (Hanwell et al., 2012) at pH 7.4 using the MMFF94 force field. The final optimization was performed in Mopac 2012 at the PM7 theoretical level, generating a file in '.pdb' format (MOPAC, 2016). Subsequently, AM1-BCC charges were assigned using UCSF Chimera 1.16, and the ligand was saved in '.mol2' format (Pettersen et al., 2004).

The protein, in '.pdb' format, was prepared using AutoDockTools version 1.5.6 (ADT) (Scripps Research Institute, San Diego, California) (Morris et al., 2009) with the addition of polar hydrogens, Kollman charges, and conversion to PDBQT format. Docking simulations were performed in AutoDock 4.2 using the Lamarckian genetic algorithm, with 100 runs and standard parameters. The active site of DNA gyrase B was defined with the grid center at (2.524; 17.272; 4.862 Å), using a 50 × 50 × 50 grid with a spacing of 0.375 Å.

As a result, binding free energy (ΔG) and inhibition constant (Ki) values were obtained, and the conformations with the lowest ΔG values were selected. The analysis of interactions between the ligand and the protein, including the types of interactions and the amino acids involved in the active site, was performed using PyMol v.3.1.3 and BIOVIA Discovery Studio (DS) Visualizer 21.1 software. Validation of the docking protocol was performed by re-docking novobiocin, with a conformation having a Root Mean Square Deviation (RMSD) value ≤ 2.0 Å considered acceptable (Bell & Zhang, 2019).

3. RESULTS

The antimicrobial activity of (R)-(+)-limonene was evaluated by determining the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) at the different concentrations specified in the methodology. It was found that the MIC of the monoterpene was 1000 µg/mL for four of the five *P. aeruginosa* strains analyzed; therefore, an MIC‰ (the lowest concentration capable of inhibiting 90% of bacterial growth) of 1000 µg/mL was determined. Regarding the MBC, it was observed that the compound also exhibited values of 1000 µg/mL for most strains, as described in Table 1.

Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the monoterpene (R)-(+)-limonene against different strains of *P. aeruginosa*

Bacterial strains	R-(+)-limonene	
	MIC (µg/mL)	MBC (µg/mL)
ATCC 9027	1000	1000
Pa43	1000	1000
Pa44	500	1000
Pa63	1000	1000
Pa68	1000	>1000

With regard to anti-adhesive activity, (R)-(+)-limonene was found to inhibit biofilm formation up to a dilution of 1:8. In turn, 0.12% chlorhexidine digluconate exhibited an inhibitory effect up to a dilution of 1:128, as shown in Table 2.

The combination of (R)-(+)-limonene with synthetic antimicrobials showed a greater synergistic effect when combined with ampicillin, gentamicin, tetracycline, ciprofloxacin, ceftazidime, and cefazolin against the strains evaluated, as shown in Table 3.

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Table 2: Minimum inhibitory concentration for adhesion (µg/mL) of the monoterpene and 0.12% chlorhexidine digluconate against the *P. aeruginosa* strain (Pa44)

					R-(+)-limonene				
µgmL	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
	-	-	-	-	+	+	+	+	+
					Chlorhexidine digluconate 0.12%				
µg/mL	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
	-	-	-	-	-	-	-	-	+

(-) No adhesion to the tube wall (+) Adhesion to the tube wall.

Table 3: Study of the association between the monoterpene (R)-(+)-limonene and synthetic antimicrobials

Antimicrobials	Association (mm)	*ATCC 9027*	*Pa43*	*Pa44*	*Pa63*	*Pa68*
AMP 10	HIATB	30	0	14	20	32
	HIATB + RL	34(↑)	12(↑)	12(↓)	22(↑)	32(*)
GEN 10	HIATB	16	18	20	26	22
	HIATB + RL	18(↑)	36(↑)	26 (↑)	26 (*)	16 (↓)
CIP	HIATB	24	30	34	30	22
	HIATB + RL	24 (*)	40 (↑)	40 (↑)	32 (↑)	24 (↑)
CAZ	HIATB	14	24	28	40	16
	HIATB + RL	18 (↑)	36 (↑)	34 (↑)	34 (↓)	12 (↓)
PEN	HIATB	22	0	0	0	22
	HIATB + RL	26 (↑)	0 (*)	0 (*)	0 (*)	20 (↓)
AMC	HIATB	34	14	20	30	38
	HIATB + RL	36 (↑)	28 (↑)	20 (*)	26 (↓)	38 (*)
AMI	HIATB	10	30	26	28	16
	HIATB + RL	14 (↑)	30 (*)	28 (↑)	28 (*)	16 (*)
LVX	HIATB	22	38	38	30	20
	HIATB + RL	22 (*)	36 (↓)	38 (*)	30 (*)	22 (↑)
NOR	HIATB	20	36	36	30	20
	HIATB + RL	22 (↑)	36(*)	38 (↑)	30 (*)	20 (*)
CFZ	HIATB	20	18	24	26	26
	HIATB + RL	24 (↑)	20 (↑)	26 (↑)	26 (*)	24 (↓)
CFL	HIATB	18	10	18	20	22
	HIATB + RL	18 (*)	10 (*)	10 (↓)	16 (↓)	20 (↓)
CRO	HIATB	24	38	38	38	30
	HIATB + RL	26 (↑)	38 (*)	38 (*)	38 (*)	36 (↑)

HIATB: inhibition zone in the presence of the antibiotic. RL: (R)-(+)-Limonene. mm: millimeter. APM10: Ampicillin. GEN10: Gentamicin. CIP: Ciprofloxacin. CAZ: Ceftazidime. PEN: Penicillin. AMC: Amoxicillin. AMI: Amikacin. LVX: Levofloxacin. NOR: Norfloxacin, CFZ: Cefazolin. CFL: Cefalotin. CRO: Ceftriaxone. (↑): Synergistic effect. (↓): Antagonistic effect. (*): Indifferent effect.

Molecular docking simulations performed in AutoDock 4.2 allowed us to evaluate the orientation and binding affinity of the ligands novobiocin (NOV) and (R)-(+)-limonene to the active site of *P. aeruginosa* DNA gyrase B, providing three-dimensional conformations, binding free energy values (ΔG), and inhibition constants (Ki). Re-docking of NOV showed higher affinity for the active site compared to (R)-(+)-limonene, in addition to an RMSD value ≤ 2 Å, confirming the reliability of the methodology (Table 4). Structural analyses revealed that the enzyme's active site is predominantly hydrophobic and that NOV establishes various stabilizing interactions, including hydrogen bonds, van der Waals interactions, pi-cationic, and hydrophobic interactions, involving key residues such as His85 and Asp83 (ligand alignment and stabilization), Arg78 (strong electrostatic interaction with the ligand's aromatic ring), Pro81, and Ile96 (ligand anchoring in the active site) (Fig. 1).

Table 4: Binding energies (ΔG) and inhibition constants (Ki) of novobiocin and (R)-(+)-limonene against the *P. aeruginosa* DNA gyrase B enzyme

Strains	Enzymes	ΔG (kcal/mol)	RMSD	K_i	ΔG (kcal/mol)	
		NOV			(R)-(+)-limonene	K_i
P. aeruginosa	DNA gyrase B	- 7.77	0.291	2.03µM	-5.22	149.13µM

(R)-(+)-Limonene exhibited a ΔG value of −5.22 kcal/mol and a Ki of 149.13 µM, indicating significant affinity for the active site of DNA gyrase B (Table 4 and Fig. 2). The 2D and 3D analyses showed that its interaction with the enzyme occurs predominantly through hydrophobic forces, van der Waals forces, and alkyl/pi-alkyl interactions, involving residues such as Val169, Val122, and Ile80 identified as critical for stabilizing the

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compound as well as Asp75, Thr167, Ser49, and Asn48, which reinforce the ligand's binding to the active site. Hydrophobicity mapping confirmed that the ligand fits into a highly hydrophobic region of the enzyme cavity, while the absence of classical hydrogen bonds suggests that the stabilization of the complex is governed primarily by hydrophobic interactions (Fig. 2).

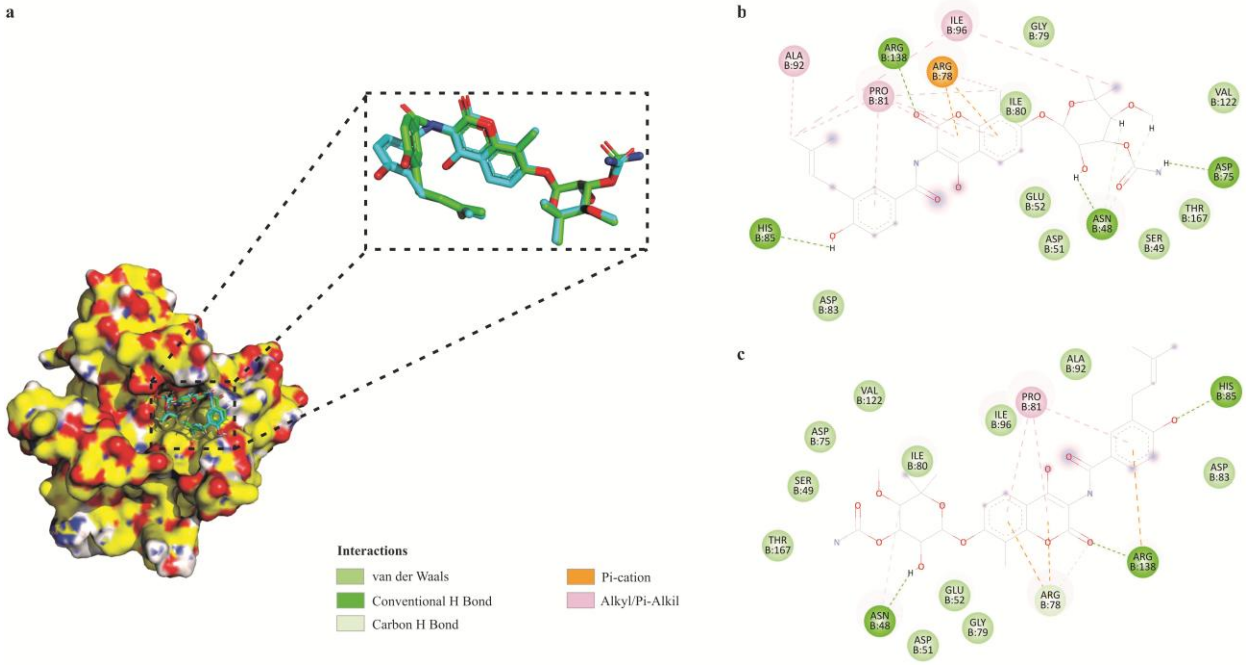

Fig. 1: Validation of the docking by re-docking of novobiocin (NOV) at the active site of *P. aeruginosa* DNA gyrase: (a) surface model of the enzyme with superimposition of the crystallographic NOV (green) and re-docking (cyan); (b) 2D interactions of NOV; (c) 2D interactions of NOV re-docking at the active site.

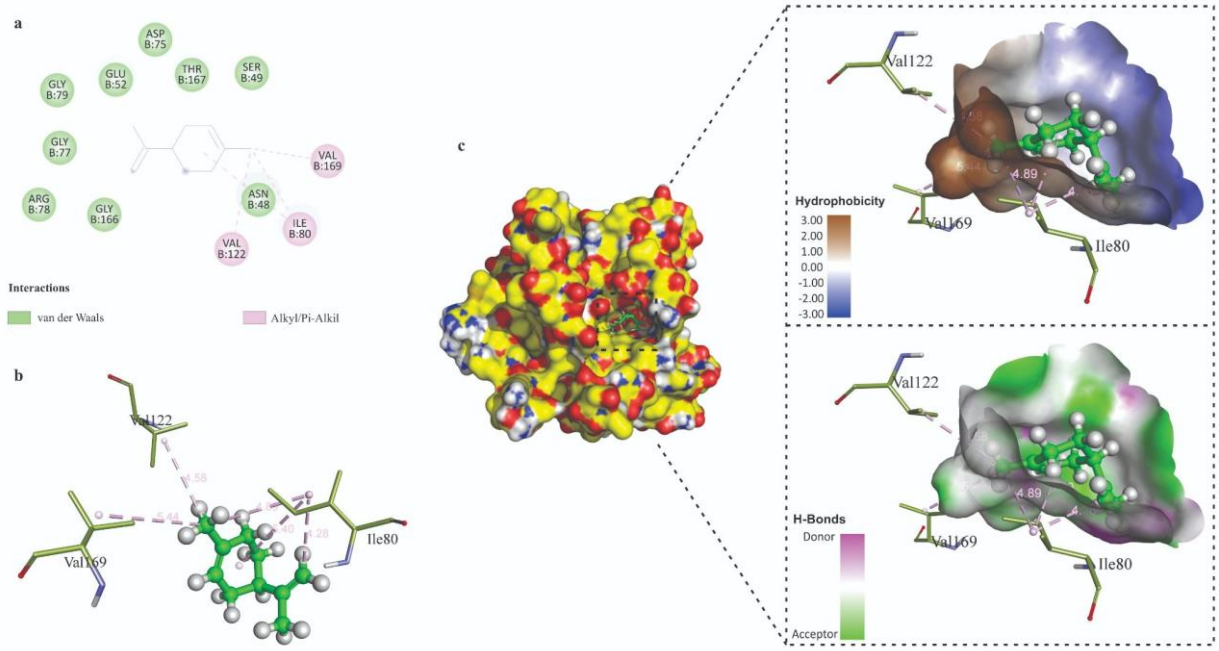

Fig. 2: Molecular docking of (R)-(+)-limonene with *P. aeruginosa* DNA gyrase B: (a) 2D interactions with key residues in the active site; (b) 3D view of the ligand–protein complex; (c) active site maps highlighting hydrophobicity and potential regions for hydrogen bonding.

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Although it is a small molecule with a limited number of functional groups, (R)-(+)-limonene exhibited binding affinity similar to that of NOV, possibly due to its high complementarity with the hydrophobic nature of the active site of DNA gyrase B. These results indicate the potential of (R)-(+)-limonene as a candidate inhibitor of this bacterial enzyme, justifying its investigation as a therapeutic alternative.

4. DISCUSSION

Antimicrobial resistance is now one of the greatest challenges facing global public health, posing a serious threat to society. In this context, the search for new antimicrobials has intensified, particularly in the fight against pathogens resistant to conventional treatments. As a result, natural products stand out as important therapeutic sources in the fight against pathogenic bacteria, due to their broad structural diversity, safety, and low toxicity (Elmaidomy et al., 2022).

Among the main microorganisms associated with this resistance scenario, *Pseudomonas aeruginosa* stands out as a nosocomial pathogen of significant clinical importance, particularly due to its high resistance to modern, potent antimicrobials and other therapeutic agents (Hassan et al., 2026).

In light of this issue, parameters such as those established by Sartoratto et al. (2004) enable evaluation of the antimicrobial potential of natural products. Minimum inhibitory concentration (MIC) values ≤500 µg/mL are considered indicative of strong antimicrobial activity; values between 600 and 1500 µg/mL indicate moderate activity, while values above 1500 µg/mL reflect weak activity. Thus, based on the results obtained, the monoterpene (R)-(+)-limonene is classified as a moderate antimicrobial agent against P. aeruginosa strains, as it exhibited an MIC_{90} of 1000 µg/mL.

In addition, the bactericidal or bacteriostatic potential of a natural product can be inferred from the MBC/MIC ratio. Ratios between 1:1 and 2:1 indicate bactericidal activity, whereas ratios greater than 2:1 suggest a bacteriostatic effect (Hafidh et al., 2011). Thus, the results obtained indicate that the compound exhibits bactericidal activity against most of the strains evaluated.

The results obtained in this study are consistent with the findings of Qaralleh (2025), who also demonstrated the potential of the monoterpene R-(+)-limonene as a multi-target pharmacological agent against clinically isolated *P. aeruginosa* from a urine sample of a patient with a urinary tract infection, emerging through antimicrobial tests as a promising alternative or adjunct to conventional antimicrobials.

The efficacy of other natural products against *P. aeruginosa* has been demonstrated in studies such as the one presented by Munira et al. (2026), who confirmed through antibacterial assays that ethanolic extracts from the root, stem bark, leaf, and fruit of *Jatropha gossypiifolia* exhibit antibacterial activity against *P. aeruginosa*, with the root extract showing greater activity compared to the others. This suggests that compounds derived from the root of J. gossypiifolia could serve as precursors for new antibacterial agents.

Furthermore, one point worth highlighting is that infections associated with biofilm formation are a major public health problem, as they can increase morbidity and mortality rates and overburden healthcare systems. Treatment of these diseases generally involves antimicrobials; however, the indiscriminate use of these drugs has contributed to bacterial resistance, underscoring the need for new therapeutic alternatives for their control (Araújo et al., 2024).

In this regard, studies involving lemon essential oil, whose main constituent is limonene, have demonstrated not only antibacterial activity but also antibiofilm activity against *Salmonella enterica* inoculated onto carrots. These results were obtained through Minimum Inhibitory Concentration (MIC) assays, crystal violet tests, and MALDI-TOF analyses, highlighting the potential of this oil due to its antimicrobial and anti-adhesive properties, which suggest possible applications in food preservation (Kačániová et al., 2024).

Studies examining the relationship between natural compounds and synthetic antimicrobials are gaining prominence, revealing potential pathways for antibacterial activity (Malczak & Gajda, 2023). In this context, a study showed that D-limonene, or R-(+)-limonene, the main constituent of the essential oils of Citrus reticulata and Citrus aurantifolia, exhibits a synergistic effect with gentamicin against clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA). These findings corroborate the results of the present study, which also demonstrated a synergistic effect for gentamicin and other antimicrobials, indicating that D-limonene may act as a modulator of the action of conventional antimicrobials, increasing their efficacy against resistant microorganisms (Sreepian et al., 2022).

It is worth noting that, although medicinal plants are an important source of bioactive compounds, their use in combination with antimicrobials requires careful evaluation to avoid undesirable antagonistic interactions. Research indicates that the indiscriminate co-administration of plant extracts with antimicrobial agents may compromise antibacterial activity rather than enhance therapeutic effects (Vishwakarma et al., 2026).

In addition to the effects observed in combination with conventional antimicrobials, studies have also shown

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that R-(+)-limonene is one of the main active constituents of the essential oils of *Sphagneticola trilobata* (leaves/stems and flowers) and that these oils exhibit significant antimicrobial and anti-adhesive activity against strains of *P. aeruginosa*. Furthermore, molecular docking analyses indicated that R-(+)-limonene has high affinity for the P. aeruginosa PqsR co-inducer binding domain, forming hydrophilic and hydrophobic interactions with conserved residues (Gln194, Leu197, Leu207, Leu208, and Ile236) and exhibiting a binding free energy of −7.38 kcal/mol. These findings reinforce the compound's antiviral and anti-adhesion potential, highlighting its strong interactions with key bacterial molecular targets (Hassan et al., 2025).

Another important therapeutic target of *Pseudomonas aeruginosa* is the enzyme studied here, DNA gyrase B, which plays a fundamental role in bacterial DNA replication and maintenance (Perera et al., 2026).

As a result, molecular docking has established itself in recent years as a fundamental element in in silico drug discovery, constituting a set of tools widely used by structural biologists and medicinal chemists. Furthermore, advances in computational methods—including approaches based on physical principles and machine learning—combined with complementary experimental techniques, have made docking an increasingly robust and efficient tool for drug discovery (Paggi et al., 2024).

5. CONCLUSION

The results obtained demonstrate that (R)-(+)-limonene exhibits moderate bactericidal and anti-adhesive activity against *P. aeruginosa* strains, with a synergistic effect when combined with synthetic antibacterials. Furthermore, predictive analyses indicated that the compound interacts with the active site of the DNA gyrase B enzyme, primarily through hydrophobic, van der Waals, and alkyl/pi-alkyl interactions. In summary, these findings reinforce the potential of (R)-(+)-limonene as a promising agent for microbial control and highlight the relevance of bioactive compounds derived from essential oils as alternatives or adjuvants for developing new therapeutic strategies in veterinary medicine.

Declarations

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Data Availability: All data generated are included in the article.

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