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Chemistry

Skin secretion of *Phyllomedusa camba* as a source of molecules against *Acinetobacter baumannii*

Secreção cutânea de *Phyllomedusa camba* como fonte de moléculas contra *Acinetobacter baumannii*

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ABSTRACT

In light of the multi-drug resistance of *Acinetobacter baumannii* to commercially available antibiotics, the search for new effective molecules against this bacterium is fundamental. In this study, metabolites present in the skin secretion of the anuran *Phyllomedusa camba* were identified using mass spectrometry. Among the molecules of interest found in the skin secretion of the anuran, 147 were tested *in silico* via molecular docking against the penicillin-binding protein and the 30S ribosome targets of *A. baumannii*. The molecular docking results indicated promising molecules against *A. baumannii*. *In vitro* assays were conducted to evaluate the antimicrobial activity of the crude skin secretion of *P. camba* against the standard strain of *A. baumannii* (ATCC 19606). The results showed antimicrobial activity for the secretion of *P. camba*, with a minimum inhibitory concentration (MIC) of 50 µg/mL and an IC_{50} of 28.65 µg/mL. Compared to results found in the literature, the obtained MIC and IC_{50} values appear promising. Bioprospecting in anurans presents itself as an alternative for the discovery of new drugs against multi-resistant bacteria.

Keywords: *Acinetobacter baumannii*; Multi-resistant bacteria; Molecular docking; Anuran

RESUMO

Diante da multirresistência de *Acinetobacter baumannii* aos antibióticos disponíveis comercialmente, é fundamental a busca por novas moléculas eficazes contra esta bactéria é fundamental. Neste trabalho, foram identificados, por espectrometria de massas, os metabólitos presentes na secreção cutânea do anuro *Phyllomedusa camba*. Das moléculas de interesse presentes na secreção cutânea do anuro, 147 foram testadas *in silico* por *docking* molecular contra os alvos poteína de ligação a penicilina e ribossomo 30S de *A. baumannii*. Os resultados de *docking* molecular apontaram moléculas promissoras contra *A. baumannii*. Foram realizados ensaios *in vitro* de atividade antimicrobiana da secreção cutânea bruta de *P. camba* contra a cepa padrão de *A. baumannii* (ATCC 19606). Os resultados mostraram atividade antimicrobiana para a secreção de *P. camba*, obtendo uma concentração inibitória minima de 50 µg/mL e IC_{50} de 28,65 µg/mL. Comparados aos resultados da literatura, os valores de concentração inibitória mínima (MIC) e IC_{50} obtidos parecem promissores. A bioprospecção em anuros se apresenta como uma alternativa na descoberta de novas drogas contra bactérias multirresistentes.

Palavras-chave: *Acinetobacter baumannii*; Bactérias multirresistentes; *Docking* molecular; Anuro

1 INTRODUCTION

Antibiotics are substances that are derived from or produced by living organisms, or even may be of synthetic origin, and are capable of inhibiting vital processes in certain microorganisms (Liu et al., 2022). Various classes of antibiotics initially showed good results against most species of pathogenic bacteria in humans. However, the indiscriminate and inappropriate use of antibiotics has favored the emergence of bacterial strains that are resistant to most modern classes of antibiotics (Cook & Wright, 2022; LaPlante et al., 2022; Zeng et al., 2022).

One bacterial species that has become a public health issue and a global concern due to its multidrug resistance to antibiotics is *Acinetobacter baumannii*. This is a Gram-negative, non-sporulating, non-glucose-fermenting, strictly aerobic, catalase-positive, oxidase-negative, and non-motile coccobacillus (Silva et al., 2021).

A. baumannii is responsible for the majority of bacterial infections in humans and can cause a variety of healthcare-associated infections (HAIs). Additionally, it has shown increasing rates of resistance to multiple antibiotics, particularly carbapenem-

resistant strains, which significantly complicates the treatment of infections caused by this bacterium (Morgan et al., 2022; Scarcella et al., 2015; Silva et al., 2021).

In 2017, the World Health Organization (WHO) published a list of priority pathogens for the development of new antibiotics, identifying *A. baumannii* as a critical priority in category 1. Furthermore, in 2021, the WHO emphasized that new antibiotics are urgently needed to treat bacterial infections (WHO, 2017; WHO, 2021).

The penicillin-binding protein (PBP), responsible for bacterial cell wall synthesis, and the 30S ribosomal subunit, responsible for protein synthesis, are well-defined targets of β-lactams and tetracyclines, respectively. These are the two main classes of antibiotics that are widely used in the treatment of various infections and are often the only alternatives available against multidrug-resistant strains (Morgan et al., 2022; Zhang et al., 2021).

1.1 Biodiversity and the development of new drugs

Biodiversity plays a crucial role in the discovery and development of new therapeutic products, serving as a natural and abundant source of bioactive molecules. In this regard, the Amazonian biodiversity stands out as a particularly noteworthy source (Ferreira, Arcanjo & Peron, 2023).

Within Amazonian biodiversity, amphibians stand out for their skin secretions, which are rich in bioactive molecules with antimicrobial properties (Indriani et al., 2023). Among the amphibians found in the Amazon, *Phyllomedusa camba* is an interesting anuran for bioprospecting due to the traditional therapeutic use of its skin secretions by indigenous tribes. These secretions exhibit antibacterial properties, including activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Pseudomonas aeruginosa* (Silva, Monteiro & Bernarde, 2019; Wu, X. et al., 2017; Wu, Y. et al., 2019).

In this context, the skin secretion of *P. camba* can be highlighted as a promising source of molecules to be tested against multidrug-resistant bacterial strains, such as carbapenem-resistant *A. baumannii*.

1.2 Bioinformatics as a tool in the discovery of new drugs

Bioinformatics tools such as virtual screening in molecular databases and molecular docking are widely used in the rational development of drugs (Naderi et al., 2019; Ranjan, Mohapatra & Das, 2020). Research involving the development of new antibacterial drugs has been facilitated by the use of molecular docking simulations (Farhan et al., 2022).

In molecular docking simulations, simplifications are applied to enable rapid computational predictions of the optimal interaction pose between a ligand, a drug candidate and a target macromolecule from a pathogen, using a genetic algorithm (Santos et al., 2020). Furthermore, based on a simplified scoring function, usually derived from binding free energies, it is possible to predict, with the aid of a positive control, whether interactions with the molecular target are favorable or not (Holcomb et al., 2022; Martins et al., 2021).

Given the urgent need for new drugs that are effective against multidrug-resistant strains of *A. baumannii*, we conducted a virtual screening via molecular docking of the molecules present in the cutaneous secretion of *P. camba* to identify potential inhibitors of the PBP or the 30S ribosomal subunit. Subsequently, *in vitro* antimicrobial activity assays were performed, which permitted the determination of the minimum inhibitory concentration (MIC) and IC_{50} values for the cutaneous secretion of *P. camba* against the standard strain of *A. baumannii* (ATCC 19606).

2 MATERIALS AND METHODS

All the computational experiments in this study were conducted on a computer equipped with a 7th-generation Intel Core i5 processor, 8 GB of RAM, 1 TB of internal storage, and an NVIDIA GeForce 940MX graphics processor.

2.1 Search for and selection of target structures

A search was conducted in the Protein Data Bank (https://www.rcsb.org/) (PDB) for crystallized protein structures of *A. baumannii* that serve as targets for β-lactam or tetracycline antibiotics, specifically penicillin-binding proteins and the 30S ribosomal subunit. Using the PDB search tool, filters were applied to focus on structures related to the species *A. baumannii*.

Parameters such as resolution, complete structure and the presence of ligands with pharmacological relevance were used for the selection of targets.

2.2 Validation redocking

To validate the molecular docking methodologies, redocking was performed on the two selected protein-ligand complexes. Preparation of the macromolecules and ligands was carried out using the AutodockTools 1.5.7 software. This included the removal of water molecules and other crystallization residues, addition of polar hydrogens and assignment of Kollman charges to the macromolecules and Gasteiger charges to the ligands. The macromolecules were kept rigid, while only the ligands were considered flexible. The side chains of the macromolecules were assigned protonation states corresponding to pH 7.4.

The grid box parameters and the Lamarckian genetic algorithm (LGA) settings for each redocking can be found in Tables 1 and 2. The AutoDock 4.2 software was used for this process.

Table 1 – Receptor macromolecules and grid box parameters

Macromolecule (PDB ID)	**Coordinates (x, y, z) [Å]**	**Dimensions (x, y, z) [Å]**
Penicilin-binding protein (3udx)	-35.185; -3.852; -12.209	42, 42, 42
70S ribosomal complex (7ryg)	278.268; 234.725; 224.485	46, 46, 46

Source: Authors (2024)

The parameters in Table 2 were sufficient to replicate the original position of the crystallized ligand within the RMSD < 2Å limits.

Table 2 – Genetic algorithm parameters

Parameter	Penicilin-binding Protein (PDB ID: 3udx)	70S Ribosomal Complex (PDB ID: 7ryg)
Number of runs	80	50
Initial population	300	300
Number of evaluations	2,500,000	2,500,000
Number of generations	27,000	27,000

Source: Authors' private collection (2024)

2.3 Bioprospecting of the molecules present in the cutaneous secretion of *P. camba*

The anuran of the *P. camba* species used to obtain the cutaneous secretion was collected in the Humaitá Forest Reserve (9°44'47.0" S, 67°40'32.0" W), located in the municipality of Porto Acre, in the state of Acre, Brazil.

2.3.1 Identification of metabolites using UHPLC/HRMS^2

The skin secretion sample from *P. camba* was collected fresh by scraping and then washing its skin with water. Access to genetic material was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under the code ADEFF4E. The Brazilian license to access the forest reserve (SISBio) was obtained under the code 73622-3, and the license, issued by the Ethics Committee Animal on Use (CEUA) of Fiocruz Rondônia, was approved under protocol 2019/20.

The sample was diluted in water to achieve 1 µg/µL. Then, 50 µL of the sample was diluted in 150 µL of MeOH, frozen, centrifuged, and the supernatant concentrated. The sample was resuspended in 50 µL of 5% MeOH with 0.1% formic acid, and 5 µg was analyzed using UHPLC/HRMS^n (Dionex Ultimate 3000/ Q-Exactive™

hybrid quadrupole-Orbitrap Plus, Thermo Fisher Scientific, Bremen, Germany) on negative and positive ionization modes in individual run analysis and in technical triplicates. Chromatography analysis was performed using a C18 column (2.1 x 50 mm, 1.8 µm, 100 Å, Zorbax Agilent), a flow rate of 0.5 mL/min at 40 ºC. Mobile phase A was composed of water with 5 mM ammonium formate and 0.1% formic acid, and mobile phase B of methanol with 0.1% formic acid. The gradient was as follows: 10% B at 0 min, 10 – 100% B from 1 to 11 min, 100% B after 16 min, and re-equilibration with 10% B from 17 to 20 min. The heated electrospray ionization (HESI) source was fixed with gas flow rates of 60 for sheath, 20 for auxiliar, and 10 for sweep, a spray voltage of 3.9 kV (positive) and -2.9 kV (negative), a current spray of 1.2 µA, 325 ºC for both capillary and auxiliar gas heater temperature, and an S-lens RF level of 80. Full mass spectrometry (MS) was acquired with a resolution of 70,000, an AGC target of 106, a maximum IT of 100 ms, a scan range of 67-1,000 m/z, and in profile mode. Fragmentation spectra were triggered in data-dependent acquisition mode with a resolution of 17,500, an AGC target of 105, a maximum IT of 50 ms, Top 20, an isolation window of 2.0 m/z, isolation offset of 0.5 m/z, stepped NCE of 10, 15 and 30, dynamic exclusion of 6 s, and in centroid mode.

Positive and negative calibrations with caffeine, MRFA, Ultramark 1,621, [M+H]+ 195 to 1,522 m/z, and SDS, sodium taurocholate, Ultramark 1621 [M-H]- 265 to 1,680 m/z (Thermo Fisher Scientific, USA) were performed before the acquisition analysis.

Data processing analysis and metabolite identification were performed with Compound Discoverer 3.3 software (ThermoScientific). Search workflow was set to untargeted mode to identify the molecules using eight databases for compound identification: Chemspider, BioCyc, mzCloud, LipidMaps, ChEMBL, HMDB, MassBank, NIST, and by setting blank samples as the chemical background. Elemental compositions and molecular weight were predicted for all the compounds.

2.3.2 Selection and download of molecules identified from *P. camba*

Among the metabolites identified from the cutaneous secretion of *P. camba*, only molecules from the classes of interest were selected for the *in silico* assays, including phenols, terpenes, alkaloids and other secondary metabolites known for their antimicrobial and herbicidal properties (Erb & Kliebenstein, 2020; PrabhakaranKutty, Meléndez & López, 2023).

The 3D structures of the selected molecules of interest were downloaded from the PubChem database.

2.4 Virtual screening using molecular docking

Using the same software, methods and parameters defined in the Validation Redocking step, virtual screening using molecular docking was performed for the molecules obtained from the cutaneous secretion of *P. camba* against the molecular targets defined in step 2.1.

2.5 Antimicrobial activity

2.5.1 Preparation of the Phyllomedusa camba cutaneous secretion solution

The crude lyophilized cutaneous secretion of *P. camba* was diluted in distilled water to a concentration of 124.3 mg/mL and subsequently re-diluted to a concentration of 400 µg/mL.

2.5.2 Preparation of the bacterial inoculum

Acinetobacter baumannii (ATCC 19606) was cultured on solid Luria Bertani agar medium for 18-24 hours at 37 °C (±2 °C). After incubation, isolated colonies were diluted in saline solution (0.9% sodium chloride, Dinâmica, Brazil) to a concentration of 0.5 McFarland ($1.5x10^8$ colony forming units - CFU/mL) and further diluted to a concentration of 10^6 CFU/mL in liquid LB medium.

2.5.3 Preparation of the chloramphenicol antimicrobial solution (reference antimicrobial)

Chloramphenicol solution was used as a reference antimicrobial. The solution was prepared by diluting the antimicrobial in 5% methanol to a concentration of 50 mg/mL and subsequently re-diluting it to a concentration of 400 µg/mL.

2.5.4 Preparation of the 96-well plate

A 96-well plate was used in duplicate. For the test samples, 100 µL of liquid LB medium was added to the wells, followed by 100 µL of *P. camba* cutaneous secretion at a concentration of 400 µg/mL in the first wells. Serial dilution was performed for the remaining wells, resulting in final concentrations ranging from 100 µg/mL to 3.13 µg/mL. For the reference antimicrobial wells, 100 µL of liquid LB medium was added, followed by 100 µL of the 400 µg/mL chloramphenicol solution. Serial dilution was also performed to obtain final concentrations ranging from 100 µg/mL to 3.13 µg/mL. To each well of the sample and the chloramphenicol, 100 µL of bacterial inoculum at a concentration of 10^6 CFU/mL was added, resulting in a final concentration of 10^5 CFU/mL in the wells. For the bacterial growth control wells, 100 µL of liquid LB medium and 100 µL of bacterial inoculum were used. As a blank, or sterility control of the experiment, wells containing 200 µL of liquid LB medium were used (Clinical & Laboratory Standards Institute [CLSI], 2020; Veiga et al., 2019).

Table 3 shows the serial dilution concentrations of the *P. camba* cutaneous secretion and the antimicrobial chloramphenicol in each well.

Table 3 – Serial dilution of *P. camba* cutaneous secretion and chloramphenicol antimicrobial

Well	**1**	**2**	**3**	**4**	**5**	**6**
	Concentration [µg/mL]					
P. camba Secretion	100	50	25	12.5	6.25	3.13
Chloramphenicol	100	50	25	12.5	6.25	3.13

Source: Authors' private collection

2.5.5 Incubation and plate reading

After preparing the plate, reading was performed using a spectrophotometer with an absorbance of 520 nm. The plate was then placed in an orbital shaker incubator set at 100 RPM, at 37 °C (±2 °C) for 18-24 hours. After incubation, a second reading was taken under similar conditions using the spectrophotometer. For analysis of the result, the absorbance data (optical density, OD) after incubation were subtracted from the pre-incubation absorbances. Bacterial growth was considered positive when the average OD values of the duplicates from each test were higher than the average OD values of the blank controls.

2.5.6 Statistical analysis of the results

To determine the MIC value, a graph was plotted with optical density values as a function of concentration for the *P. camba* cutaneous secretion sample and the reference antimicrobial, chloramphenicol.

To determine the IC_{50} of the *P. camba* cutaneous secretion, the GraphPad Prism software was used, whereby the optical density values were normalized to the percentage of inhibition, and a sigmoidal curve fitting was applied (Farias et al., 2012).

3 RESULTS AND DISCUSSION

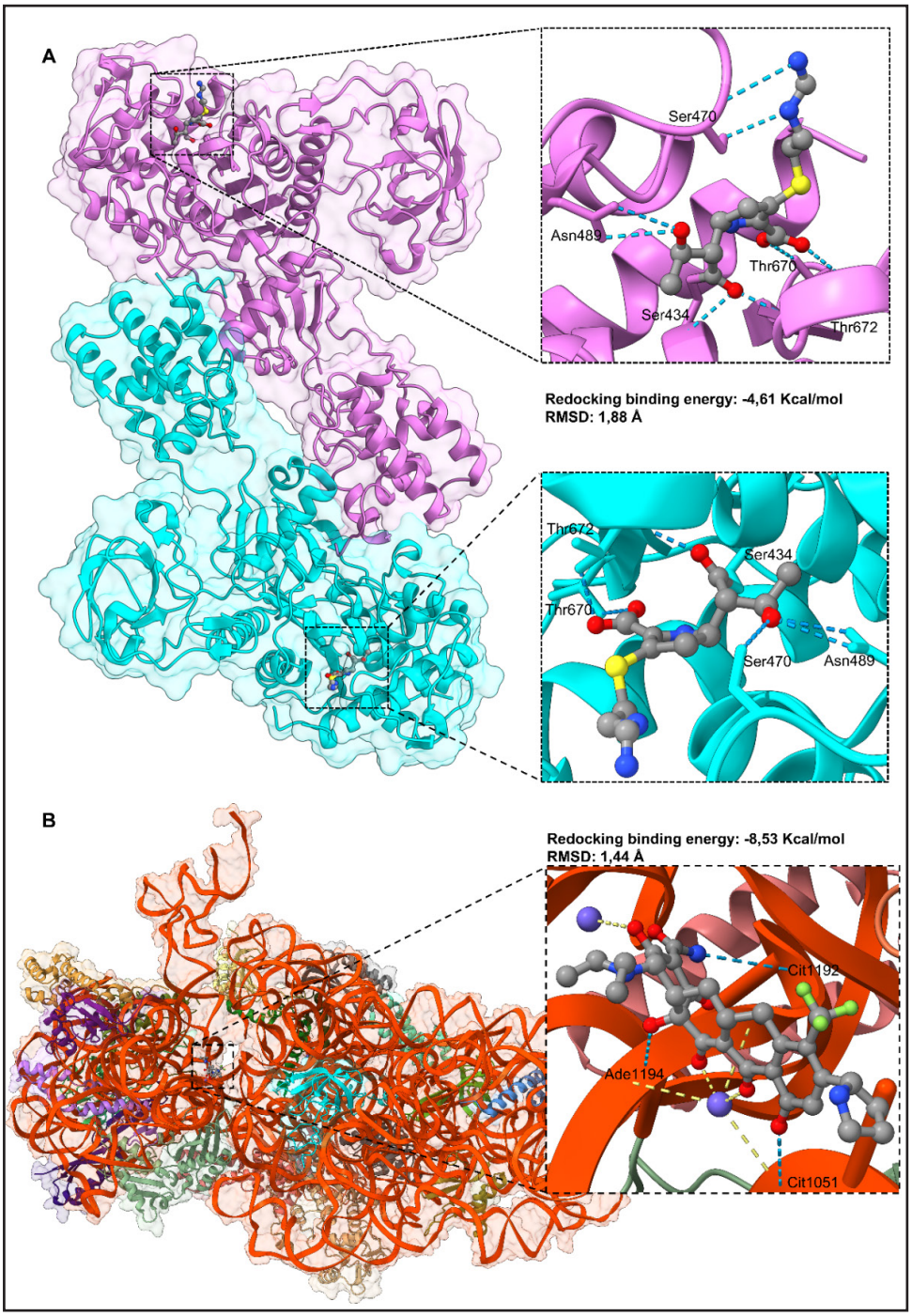
3.1 Target selection

For the penicillin-binding protein, a total of 5 structures were found in the PDB. For the *A. baumannii* ribosome, a total of 15 structures were identified.

For the penicillin-binding protein, the structure with PDB code 3UDX was selected, with a resolution of 2.5 Å. This crystal structure of PBP1a is in complex with imipenem (Figure 1A), a well-established carbapenem used in the treatment of infections caused by Gram-negative bacteria (Motsch et al., 2020).

For the ribosome, the structure with PDB code 7RYG was selected, with a resolution of 2.38 Å. This structure represents the 70S ribosome, with the small 30S subunit in complex with tetracycline TP-6076 (Figure 1B), a new and promising synthetic antibiotic developed by Tetraphase Pharmaceutics (Rusu & Buta, 2021; Seifert et al., 2020).

Figure 1 – Molecular structures of the *A. baumannii* targets. A) 3UDX and B) 7RYG



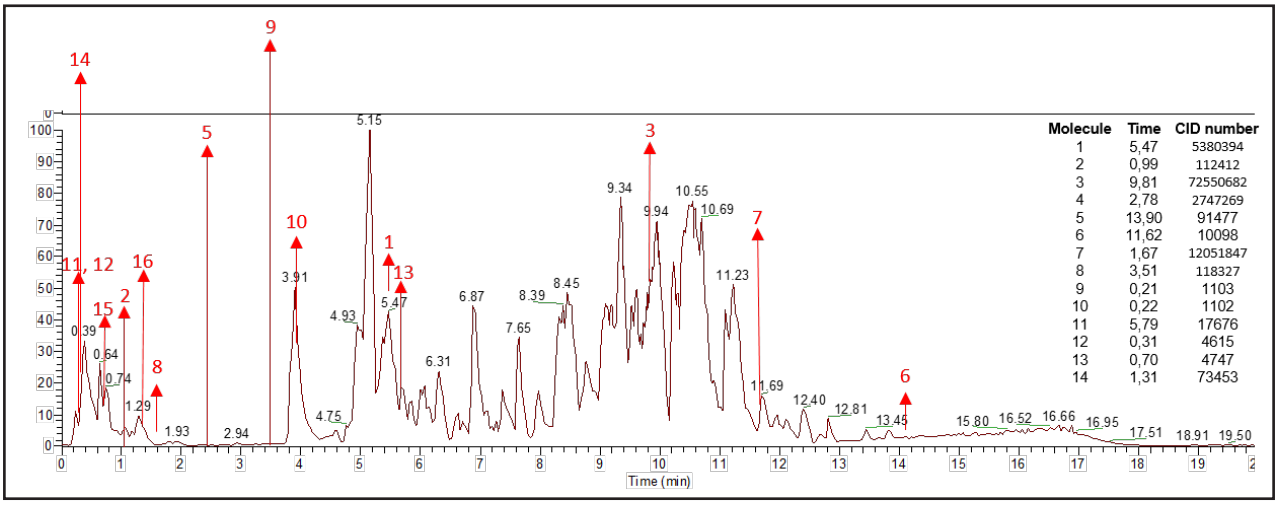
Source: Authors (2024). Legend: A: Penicillin-binding protein dimer with imipenem bound at its active site. B: 30S ribosomal subunit with tetracycline TP-6076 at its active site. Portions of nucleotides in orange, and protein chains in different colors. Hydrogen bonds in cyan, and coordinated metal bonds in light yellow

3.2 Bioprospecting of the molecules present in the cutaneous secretion of *P. camba*

From the molecules identified in *P. camba* cutaneous secretion using mass spectrometry, amino acids, peptides, amino acid derivatives, repeated molecules, and molecules with mass values differing from their actual value were excluded, resulting in a total of 169 molecules of interest. Of these, 144 molecules had their 3D structures deposited in the PubChem database. The list of these 144 molecules obtained from the PubChem database can be found in Table S1 in the Supplementary Material.

Figure 2 shows the mass spectrometry graph of the P. camba cutaneous secretion, with the identification of the molecules of interest.

Figure 2 – Chromatogram based on mass spectrometry analysis in positive ionization. The arrows indicate the elution of the metabolites that showed relevant docking energy, described in Tables 4 and 5



Source: Authors (2024). Legend: Mass spectrum in red, with time values in minutes. The red arrows indicate the peaks corresponding to the compounds of interest. Next to it, a table with the CID corresponding to each molecule identified in the spectrum

3.3 Virtual screening using molecular docking

Table 4 presents the molecular docking results of the ten molecules with the best binding energies to the penicillin-binding protein of *A. baumannii*. Compared to the control binding energy of -4.61 kcal/mol obtained from the redocking of imipenem

with PBP1a, a total of 80 molecules out of the 146 screened showed favorable binding energies against PBP. The list with the binding energy ranking of the 146 molecules can be found in Table S2 in the supplementary material.

Table 4 – Molecules obtained with the best molecular docking results against *A. baumannii* PBP1a

(To be continued...)

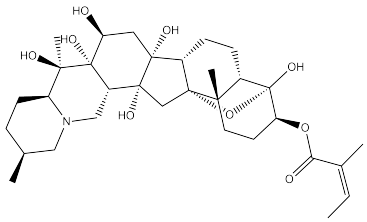
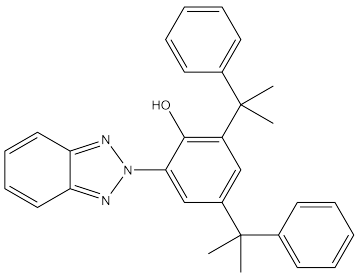
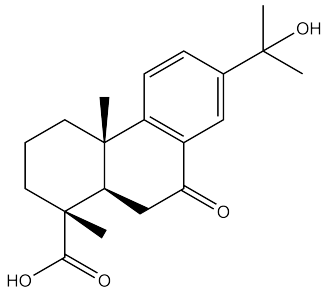
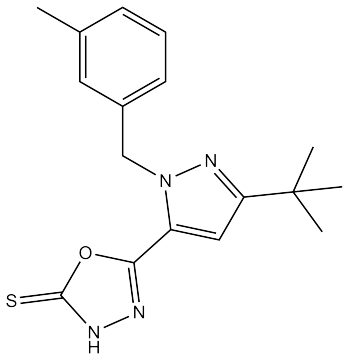
Name	CID	Molecular Structure	Molecular Formula	Binding Energy [kcal/mol]
Cevadine	5380394		$C_{32}H_{49}NO_9$	-9.22
2-(benzotriazol-2-yl)-4,6-bis(2-phenylpropan-2-yl) phenol	112412		$C_{30}H_{29}N_3O$	-8.66
(1R,4aS)-7-(2-hydroxypropan-2-yl)-1,4a-dimethyl-9-oxo-3,4,10,10a-tetrahydro-2H-phenanthrene-1-carboxylic acid	72550682		$C_{20}H_{26}O_4$	-8.33
5-[5-tert-butyl-2-[(3-methylphenyl)methyl] pyrazol-3-yl]-3H-1,3,4-oxadiazole-2-thione	2747269		$C_{17}H_{20}N_4OS$	-7.50

Table 4 – Molecules obtained with the best molecular docking results against *A. baumannii* PBP1a

(To be continued...)

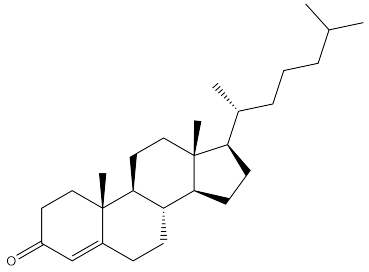
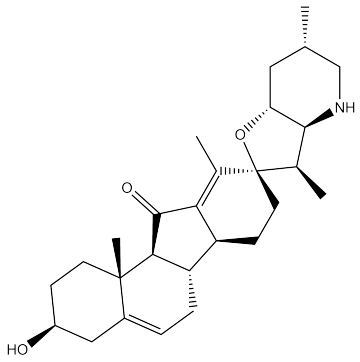
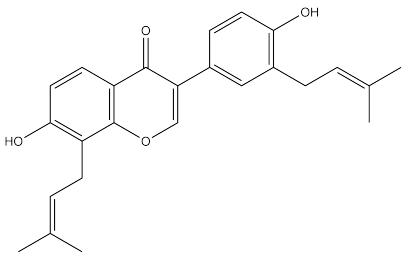
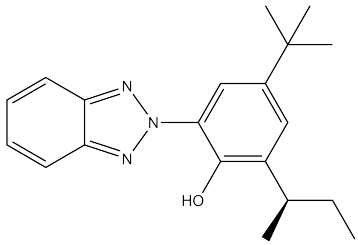
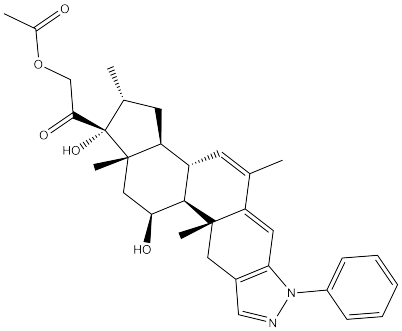
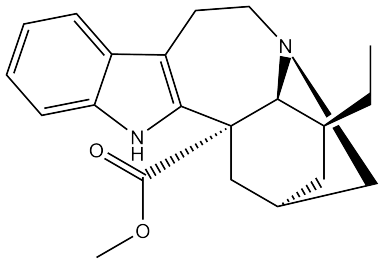
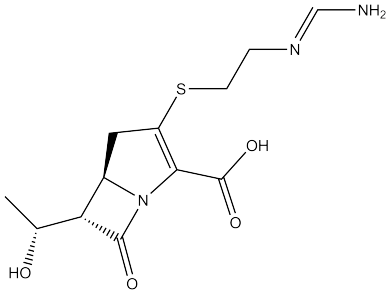
Name	CID	Molecular Structure	Molecular Formula	Binding Energy [kcal/mol]
Colestenone	91477		$C_{27}H_{44}O$	-7.45
Jervine	10098		$C_{27}H_{39}NO_3$	-7.31
Erysubin F	12051847		$C_{25}H_{26}O_4$	-7.28
2-(benzotriazol-2-yl)-6-butan-2-yl-4-tert-butylphenol	118327		$C_{20}H_{25}N_{3O}$	-7.23
Cortivazol	66249		$C_{32}H_{38}N_2O_5$	-7.08

Table 4 – Molecules obtained with the best molecular docking results against *A. baumannii* PBP1a

(Conclusion)

Name	CID	Molecular Structure	Molecular Formula	Binding Energy [kcal/mol]
Coronaridine	6426909		$C_{21}H_{26}N_2O_2$	-7.08
Imipenem (control)	104838		$C_{12}H_{17}N_3O_4S$	-4.61

Source: Authors (2024)

The molecule identified with CID 5380394, known as cevadine or veratrine, exhibited the best binding energy against the penicillin-binding protein. Cevadine is a natural alkaloid found in the plant species *Schoenocaulon officinale* and *Veratrum oblongum* (PubChem) (https://pubchem.ncbi.nlm.nih.gov/compound/5380394). Cevadine, present in the extract of *Schoenocaulon officinale*, is recognized as a natural insecticide, and its photodegradability makes it environmentally friendly (Pavarini et al., 2023; Qu et al., 2022; Silva, Sato & Raga, 2019).

Observing the molecules in Table 4, a trend can be noted in the presence of cyclic groups, often fused with other rings, containing nitrogen as a heteroatom. This feature may be relevant for interactions with the penicillin-binding protein.

Against the 30S ribosomal subunit of *A. baumannii*, a total of 10 molecules demonstrated favorable binding energies, i.e., with binding energies lower than the control value of -8.53 kcal/mol obtained from the redocking of TP-6076. Table 5 presents the 10 molecules with the best binding energies to the 30S ribosomal target.

The list with the binding energy ranking of the 146 molecules can be found in Table S3 in the supplementary material.

Table 5 – Molecules obtained with the best molecular docking results against the *A. baumannii* 30S ribosomal subunit

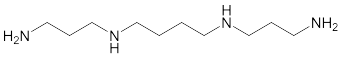
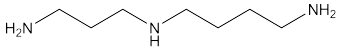
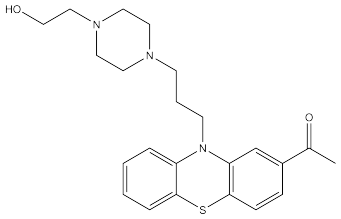
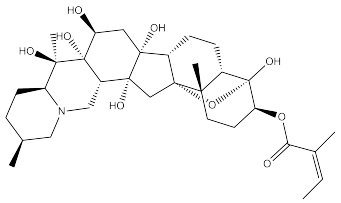
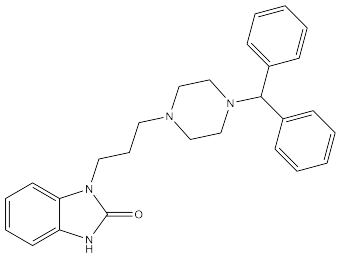
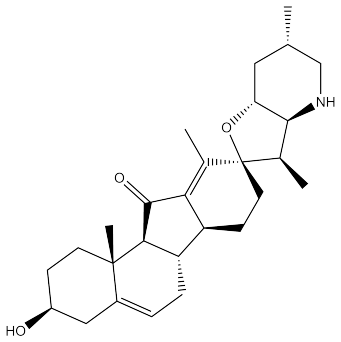
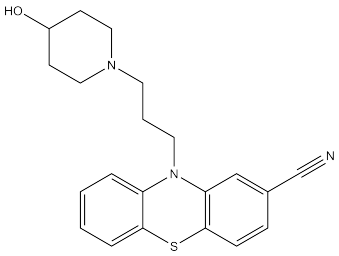
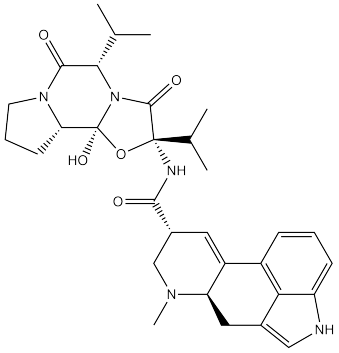
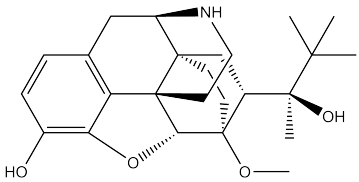
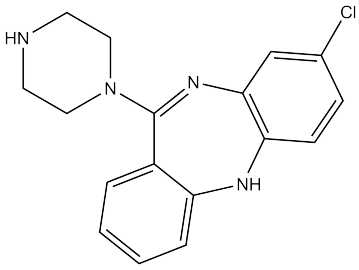
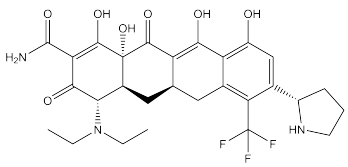
Name	CID	Molecular Structure	Molecular Formula	Binding Energy [kcal/mol]
Espermine	1103		$C_{10}H_{26}N_4$	-13,59
Espermidine	1102		$C_7H_{19}N_3$	-12,41
Acetophenazine	17676		$C_{23}H_{29}N_3O_2S$	-11,51
Cevadine	5380394		$C_{32}H_{49}NO_9$	-10,51
Oxatomide	4615		$C_{27}H_{30}N_4O$	-10,40
Jervine	10098		$C_{27}H_{39}NO_3$	-10,38

Table 5 – Molecules obtained with the best molecular docking results against the *A. baumannii* 30S ribosomal subunit

Name	CID	Molecular Structure	Molecular Formula	Binding Energy [kcal/mol]
Periciazine	4747		$C_{21}H_{23}N_{3O}S$	-9,22
Ergocornine	73453		$C_{31}H_{39}N_5O_5$	-8,64
Norbuprenorphine	114976		$C_{25}H_{35}NO_4$	-8,64
Desmethylclozapine	135409468		$C_{17}H_{17}ClN_4$	-8,57
TP-6076 (control)	162423126		$C_{28}H_{32}F_3N_3O_7$	-8,53

Source: Authors' private collection (2024)

Observing the results in Table 5, it can be noted that the two molecules with the best binding energies in the ranking are CID 1103 and CID 1102, which are spermin and spermidine, respectively. Spermin is a polyamine formed from spermidine. Spermidine is a polyamine involved in stabilizing the 30S ribosomal subunit during protein synthesis in bacteria, as demonstrated by Belinite et al. (2021), in which spermidine is used to provide flexibility and stabilize helix 44 in the protein synthesis process in *S. aureus*. The fact that the molecular docking experiment reached this expected result, despite spermin and spermidine not having shown potential against *A. baumannii*, demonstrates the reliability of the docking methodology used, as it was able to mimic a natural interaction phenomenon between polyamines involved in stabilizing the 30S ribosomal subunit.

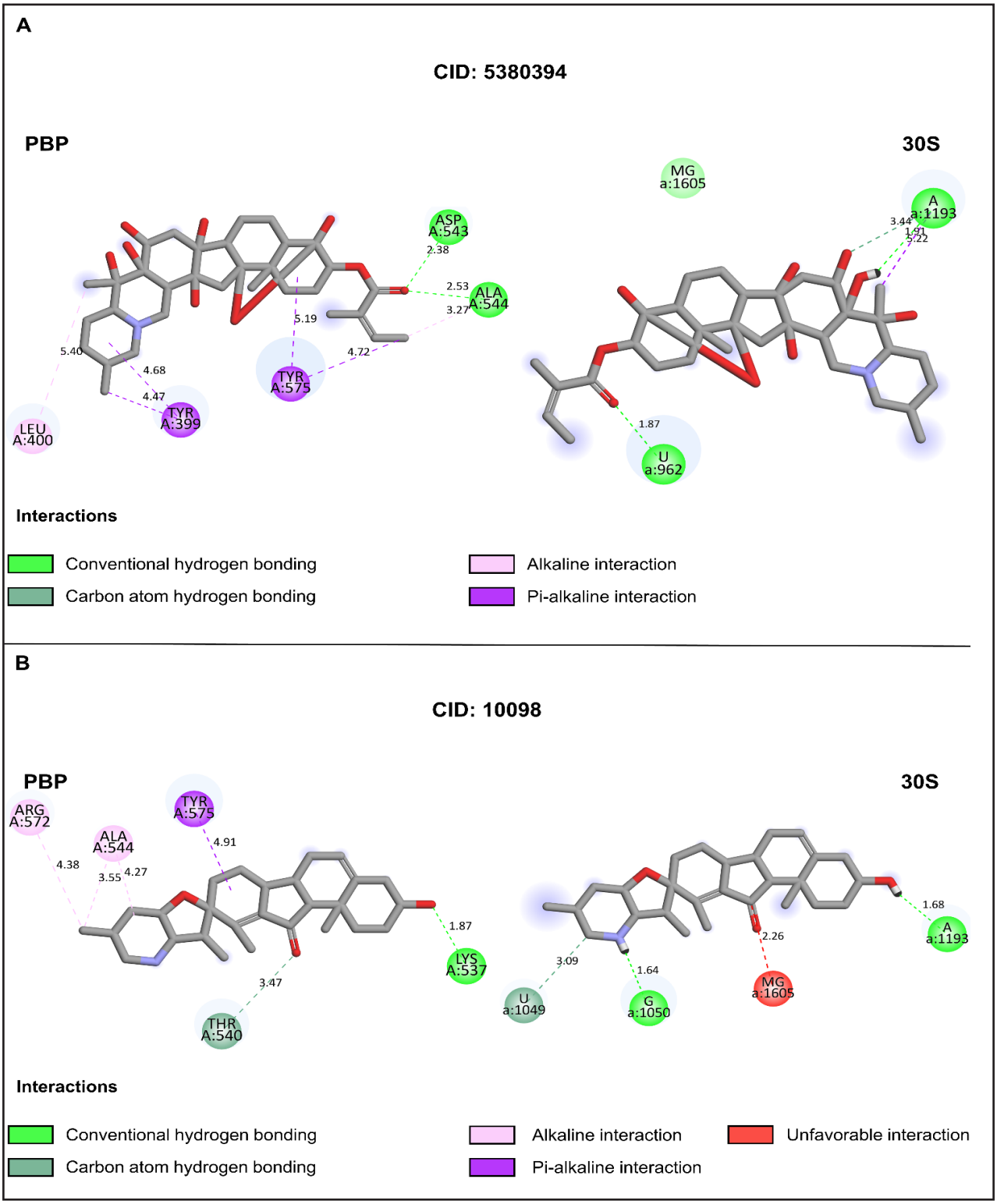
In fourth place in the ranking against the 30S ribosomal subunit, we again observe cevadine, which appears to be a promising molecule to be tested against *A. baumannii*, as it ranks among the top ten molecules against both targets, PBP and 30S.

In sixth place in the ranking against the 30S ribosomal subunit is the molecule CID 10098, jervine, which also appears among the top ten molecules in the binding energy ranking against PBP. This suggests its potential to be tested against *A. baumannii*.

In Figure 3, the interactions with the PBP1a and 30S targets for the molecules cevadine (CID 5380394) and jervine (CID 10098) are shown. These two molecules appear among the top ten binding energies for both targets.

For the docking pose of cevadine with PBP1a (Figure 3A), none of the interactions typical of the binding mode between β-lactams and penicillin-binding proteins are present, which was already expected since cevadine is an alkaloid and does not possess the same characteristics as a β-lactam (Moon et al., 2018). A prevalence of Pi-alkaline interactions involving the Pi-electrons of the cevadine rings is observed.

Figure 3 – Docking poses of the A) CID 5380394 and B) CID 10098 molecules against PBP1 and 30S



Source: Authors (2024). Legend: A: Docking poses of CID 5380394 molecule with PBP1a and 30S ribosomal subunit, respectively. B: Docking poses of CID 10098 molecule with PBP1a and 30S ribosomal subunit, respectively. Intermolecular interactions are represented by dashed lines of different colors: Conventional hydrogen bond (light green), carbon atom hydrogen bond (dark green), alkaline interaction (light purple), Pi-alkaline interaction (violet), and unfavorable interaction (red)

For the docking pose of cevadine (Figure 3A) with the 30S ribosomal subunit, a greater similarity with the interaction mode of tetracyclines to the 30S is observed, most likely due to the higher structural similarity of cevadine with tetracyclines. Interactions with uracil 962 and adenine 1193 are present, as confirmed by Zhang et al. (2021) and Morgan et al. (2022).

In the docking pose of jervine with PBP1a (Figure 3B), hydrogen bonds with threonine 540 and lysine 537 are observed, which are common to the binding mode of β-lactams to penicillin-binding proteins (Moon et al., 2018). In its docking pose with the 30S ribosome, interactions with guanine 1050 and adenine 1193, confirmed by Morgan et al. (2022), are observed, in addition to an interaction with uracil 1049 and a repulsion with the magnesium ion.

3.4 Antimicrobial activity

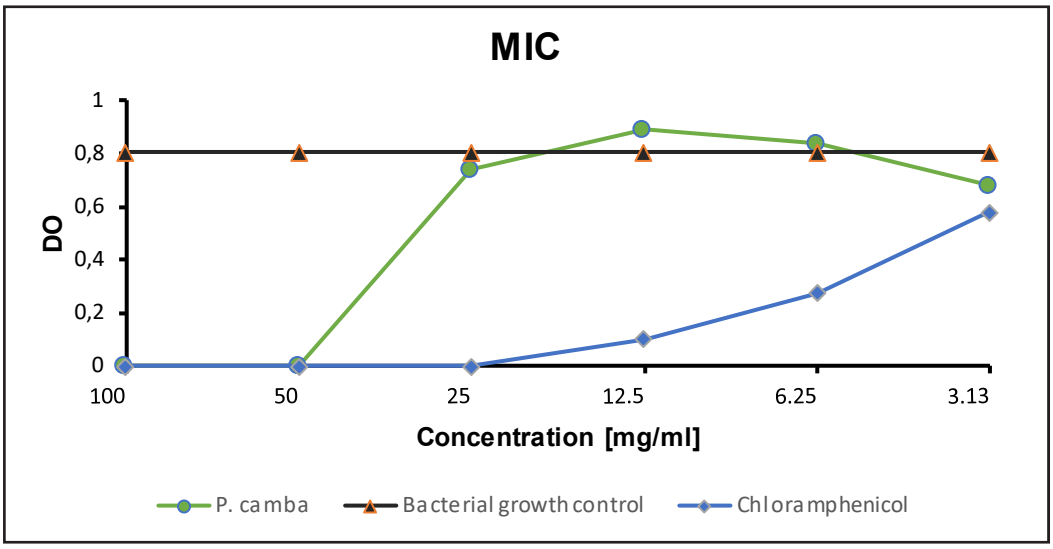
In Figure 4, the results of the MIC assay for the skin secretion of *P. camba* can be seen. The optical density (absorbance) is presented as a function of the sample concentration. An increase in the turbidity of the liquid content in the wells of the plate was observed, starting from a concentration of 25 µg/mL of the skin secretion of *P. camba*. In this regard, the MIC of *P. camba* secretion for *A. baumannii* (ATCC 19606) is expressed as 50 µg/mL. For the reference antimicrobial, chloramphenicol, a MIC value of 25 µg/mL is observed.

Nalbantsoy et al. (2016) tested the crude skin secretion of *Bufo bufo*, *Bufo verrucosissimus* and *Bufotes variabilis* rich in alkaloids, against Gram-negative bacteria and determined MIC values between 125 µg/mL and 250 µg/mL. When compared to these values from the literature, the MIC result of 50 µg/mL obtained for the skin secretion of *P. camba* against *A. baumannii* appears promising and indicates antimicrobial effectiveness.

By fitting the data to the sigmoidal curve in the GraphPad Prism software (Figure 5), the IC_{50} value for the skin secretion of *P. camba* and for the antibiotic chloramphenicol

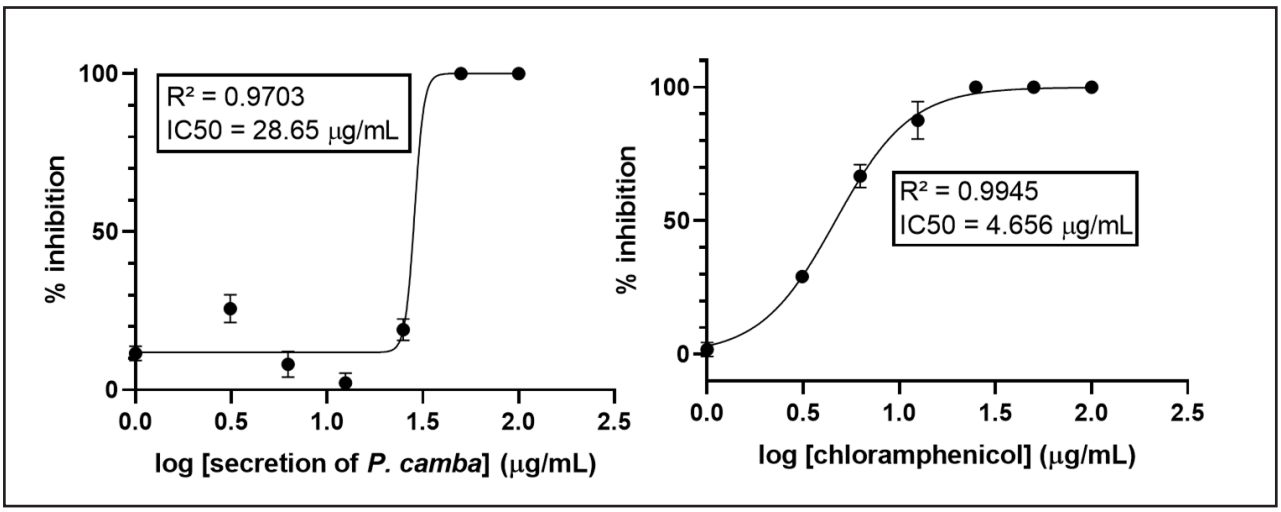
was determined. With a 95% confidence interval and a correlation coefficient of 0.9703 for the sigmoidal curve fit, an IC_{50} value of 28.65 µg/mL was obtained for the skin secretion of *P. camba*. In comparison, for the chloramphenicol, an IC_{50} value of 4.656 µg/mL was obtained.

Figure 4 – MIC curves for the sample, chloramphenicol and bacterial growth control



Source: Authors (2024). Legend: Absorbance as a function of concentration for the sample, bacterial growth control and chloramphenicol

Figure 5 – Fitting the experimental data to the sigmoidal curve



Source: Authors (2024). Legend: Inhibition percentage as a function of the logarithm of concentration. The graph of the sample is on the left, and the graph of the chloramphenicol is on the right

The promising *in vitro* results obtained for the cutaneous secretion of *P. camba* demonstrated efficacy only against the standard strain of *A. baumannii* (ATCC 19606), and therefore do not allow for conclusions regarding its effectiveness against multidrug-resistant strains. Another limitation of the study was the lack of isolation and testing of purified compounds present in the cutaneous secretion of *P. camba*, which may exhibit different MIC and IC□□ values. This would enable a more accurate identification of bioactive compounds with antimicrobial potential, being assisted by the *in silico* results in the screening of the most likely inhibitors.

Thus, future studies should include *in vitro* assays using the purified molecules from the cutaneous secretion of *P. camba*, tested both against the standard strain (ATCC 19606) and multidrug-resistant clinical isolates of *A. baumannii*. Furthermore, complementary cytotoxicity and *in vivo* studies are required to provide a more robust foundation for the therapeutic potential of these compounds as candidates for drug development against *A. baumannii*.

4 FINAL CONSIDERATIONS

The molecules, obtained through the bioprospecting of the cutaneous secretion of *P. camba,* yielded promising results in the molecular docking phase. Additionally, the results of the *in vitro* antimicrobial activity assay of the *P. camba* secretion suggest antimicrobial potential against *A. baumannii*, indicating a promising source of molecules with antimicrobial properties.

It should be remembered that biodiversity holds a vast collection of biomolecules with potential activities of interest to be studied and it plays a significant role in drug development. Each year, the number of drugs produced from natural products increases, as does the number of crude extracts, enriched extracts, bioactive molecule data. In this regard, the promising *in silico* and *in vitro* results obtained in this study for the cutaneous secretion of *P. camba*, which align with the data in the literature on its antimicrobial activity, are likely to spark even greater interest in *P. camba*.

The present study may contribute to future research, given the need for *in vitro* and *in vivo* assays with purified molecules from *P. camba* against multidrug-resistant strains of *A. baumannii*.

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