DISCIPLINARUM SCIENTIA *Disciplinarum Scientia.* **Série: Naturais e Tecnológicas**
Santa Maria, v. 25, n. 3, p. 19-28, 2024
Recebido em: 14.02.2024. Aprovado em: 0.11.2024.
ISSN 2176-462X | DOI: doi.org/10.37779/nt.v25i3.4809

IN-SILICO ANALYSIS OF NATURAL COMPOUNDS FOR COMBATING COLISTINRESISTANT BACTERIA

ANÁLISE IN-SILICO DE COMPOSTOS NATURAIS PARA COMBATE A BACTÉRIAS RESISTENTES À COLISTINA[1]

Eduarda Dezanet Trindade[2], Mirkos Ortiz Martins[3] e Bruno Stefanello Vizzotto[4]

ABSTRACT

Bacterial resistance is a global issue causing medical complications and increased mortality. The mcr-1 gene, which confers resistance to colistin, is a key factor in the spread of this resistance. To combat multidrug-resistant microorganisms, new approaches like phytotherapy, utilizing medicinal plants like Aloe vera and Origanum vulgare, are needed. Computational simulations can expedite research, but experimental studies are crucial for ensuring predictive results. Our study analyzed the in-silico effectiveness of Aloe emodin and carvacrol compounds against MCR-1 protein responsible for colistin-resistant in bacteria. Results showed high binding affinities with the protein, making them excellent phototherapeutics to be used against infections caused by colistinresistant bacterial pathogens. Further research is needed to further understand these results.

Keywords: *Phytotherapy; Molecular Docking Simulation; Polymyxin E; Antimicrobial Resistance.*

RESUMO

A resistência bacteriana é um problema global que causa complicações médicas e aumento da mortalidade. O gene mcr-1, que confere resistência à colistina, é um fator chave na propagação dessa resistência. Para combater microrganismos multirresistentes, são necessárias novas abordagens como a fitoterapia, utilizando plantas medicinais como *Aloe vera* e *Origanum vulgare*. Simulações computacionais podem acelerar a pesquisa, mas estudos experimentais são cruciais para garantir resultados preditivos. Nosso estudo analisou a eficácia *in silico* dos compostos aloe emodina e carvacrol contra a proteína MCR-1 responsável pela resistência à colistina em bactérias. Os resultados mostraram altas afinidades de ligação com a proteína, tornando-os excelentes fitoterápicos a serem usados contra infecções causadas por patógenos bacterianos resistentes à colistina. Mais pesquisas são necessárias para entender melhor esses resultados.

Palavras-chave: Fitoterapia; Polimixina E; Resistência Antimicrobiana; Simulação de Docking Molecular.

1 Trabalho Final de Graduação

2 Graduanda em Biomedicina - Universidade Franciscana - UFN, Santa Maria, RS. E-mail: eduarda.dezanet@gmail.com. ORCID: https://orcid.org/0000-0001-7930-4645

3 Coorientador. Docente do Programa de Pós-Graduação em Nanociências - Universidade Franciscana - UFN, Santa Maria, RS. E-mail: mirkos@gmail.com. ORCID: https://orcid.org/0000-0002-3983-1624

4 Orientador. Laboratório de Pesquisa em Microbiologia Molecular, Universidade Franciscana - UFN, Santa Maria, RS. E-mail: bvizzotto@yahoo.com.br. ORCID: https://orcid.org/0000-0001-6819-4081

INTRODUCTION

Bacterial resistance refers to the ability that microorganisms have acquired to survive to doses of antibiotics that would typically be lethal to them. Bacterial resistance is a global concern, as treatments for these infections become ineffective, leading to medical complications, increased healthcare costs, and consequently, elevated mortality (DUNN *et al.*, 2020). To tackle bacterial resistance, it is essential to promote the rational use of antibiotics, implement effective infection prevention practices, and raise awareness among healthcare professionals and the public about the importance of responsible antibiotic use. Acquired resistance genes are obtained through genetic recombination via transformation, conjugation, and bacterial transduction. Antibiotic resistance genes act through diverse mechanisms, such as altering penicillin-binding proteins, enzymatic inactivation of β-lactamases, and reducing the permeability of the bacterial cell wall (BLOT *et al.*, 2019).

The mcr-1 gene is responsible for conferring resistance to colistin, an antibiotic used as a last resort in the treatment of microorganisms that produce carbapenems (KPC), meaning they are resistant to carbapenem antibiotics. The presence of these microorganisms in hospital environments and communities can lead to the spread of resistance to other microorganisms, making their control on a broader scale even more challenging (LIU *et al.*, 2016). The mcr-1 gene is of the plasmid type, meaning it can easily transfer between bacteria of different species. This gene encodes a member of the phosphoethanolamine transferase (PEA) family that decorates the lipid A heads of lipopolysaccharides, altering the 1’ and 4’ phosphate groups of lipid A, neutralizing the negative charge of lipopolysaccharides, and reducing binding with colistin, which carries a positive charge (LIU *et al.*, 2016, SON *et al.*, 2019).

Due to the lack of effectiveness of conventional treatments for bacterial infections, which rely on the use of antimicrobial agents, it becomes essential to adopt new therapeutic approaches in the fight against multidrug-resistant microorganisms. Phytotherapy, which employs medicinal plants in treatments, emerges as a viable therapeutic alternative to combat antibiotic-resistant bacteria, thanks to its therapeutic properties such as anti-inflammatory, antiviral, antibacterial, among others (FREIRES & JUNIOR, 2022). One of the plants exhibiting these characteristics is known as Aloe vera. Due to the widespread availability of this plant in Brazil and its lower toxicity compared to other medicinal plants, it causes fewer side effects while maintaining antimicrobial activity. Among its active components is Aloe emodin (AE), an anthraquinone and natural dye. This compound stands out for its role as a photosensitizer, owing to its photophysical and photochemical properties favorable to this alternative treatment (LI, BEUERMANN & VERMA, 2020, FILHO *et al.*, 2022). Another medicinal plant that exhibits these and other essential characteristics for phytotherapy is Origanum vulgare, which is widely used in culinary applications as a seasoning but contains active compounds that confer antioxidant, antimicrobial, and anti-inflammatory properties. The active principal present

on the plant carvacrol, stand out for their photosensitive properties that are favorable for phototherapy treatment (LAGHA *et al.*, 2019).

To conduct research on new treatments more rapidly and to understand molecular interactions, computational simulation is one of the best resources to be employed. As it allows for experiments with large volumes of data in a short period of time, compared to laboratory bench experiments, this method aids the researcher in understanding the mechanisms of molecular action in association before in vitro assays, enhancing the efficiency of practices and reducing research time and input costs. However, while simulation provides predictive results, experimental studies are essential to ensure the reliability and applicability of these findings (TORRES *et al.*, 2019). Hence, new approaches are necessary for the treatment of resistant bacteria with phytotherapy, using computational simulation to expedite research. Thus, this study aims to analyze the bind efficiency of the compounds AE and carvacrol, against MCR-1 protein, responsible for colistin resistant bacteria, to be used as adjuncts in colistin treatments.

METHODS

DETERMINATION OF THE MCR-1 PROTEIN

For the molecular docking assays, we selected the protein structure corresponding to the catalytic domain of the MCR-1 protein from Escherichia coli (PDB Code: 5GOV) [10]. According to their findings, the active site of phosphoethanolamine transferase is located at threonine residue 285 of the A chain of the protein (THR 285 A) and has 11 zinc atoms distributed throughout the molecule.

The protein has this conformation as the crystal structure of phosphoethanolamine transferase A (LptA) from Neisseria meningitidis (PDB ID: 4KAY) was used as a template. After model reconstruction and refinement, the three-dimensional structure of the catalytic domain of the MCR-1 protein was determined. The structure exhibits this conformation due to flexible α and β chains (HU *et al.*, 2016). The residues present are T198 to I540, with the active site being threonine residue 87, because this structure represents approximately 40% of the total catalytic domain of the protein, which is the extracellular domain.

LIGAND SELECTION

The chosen natural compounds were AE and carvacrol. Their three-dimensional structures were retrieved from the PubChem database with IDs 10207 and 10364, respectively (LAGHA *et al.*, 2019, CHEN *et al.*, 2022). These compounds were selected as they are active ingredients in medicinal plants with antimicrobial properties, making them excellent candidates for antimicrobials. Additionally, they possess photosensitive properties, crucial for an effective and safe photosensitizer.

MOLECULAR DOCKING

It is of paramount importance to use software to assess the binding of the ligand structure to the MCR-1 protein, as it is a key tool for predicting the best fitting orientation of a ligand in a protein. This approach allows us to characterize the behavior of small molecules at the binding site of target proteins and elucidate molecular interactions (TROTT & OLSON, 2010).

The software used was AMDock: AutoDock Zn, given that the utilized protein contains zinc in its structure. To achieve the best result, at the project's initiation, it is necessary to determine the coordinates and size of a box, where various ligand positions relative to the protein will be tested. The chosen coordinates were 24.8 x -13.2 x -42 Å, with the box size in all directions set to 25 Å, and the pH for analysis was 7.4. These coordinates were selected because the active site residue of the protein is located at the center of this box.

MOLECULAR VISUALIZATION

For the analysis of the results from the docking process conducted by AutoDock, it is essential to use the Biovia Discovery Studio Visualizer software. This application enables the visualization of the ligand's position in relation to the protein. Additionally, it offers the advantage of generating a two-dimensional diagram illustrating the interactions between the ligand and protein residues. In the software, it is possible to highlight chosen protein residues in the three-dimensional molecule and examine intermolecular bonds in a two-dimensional diagram (TORRES *et al.*, 2019).

RESULTS AND DISCUSSION

Three-dimensional structures of the compounds were selected from the PubChem database, and the crystallized structure of the MCR-1 protein was obtained from the PDB Databank. The crystallized structure consists of two chains, A and B, composed of flexible β chains and α chains, forming a sandwich-like conformation. A zinc atom is located near threonine 87 residue, providing an electronegative potential to attract and bind the protein to the NH3 terminal of the lipid A in the bacterial cell wall, conferring resistance to the bacterium.

The protein's active site is in a cavity, facilitating it's binding to the bacterium. We analyzed which representation is more suitable for visualizing the correlation between threonine 87 residue and the compound, either the 3D ribbon or the solid surface form.

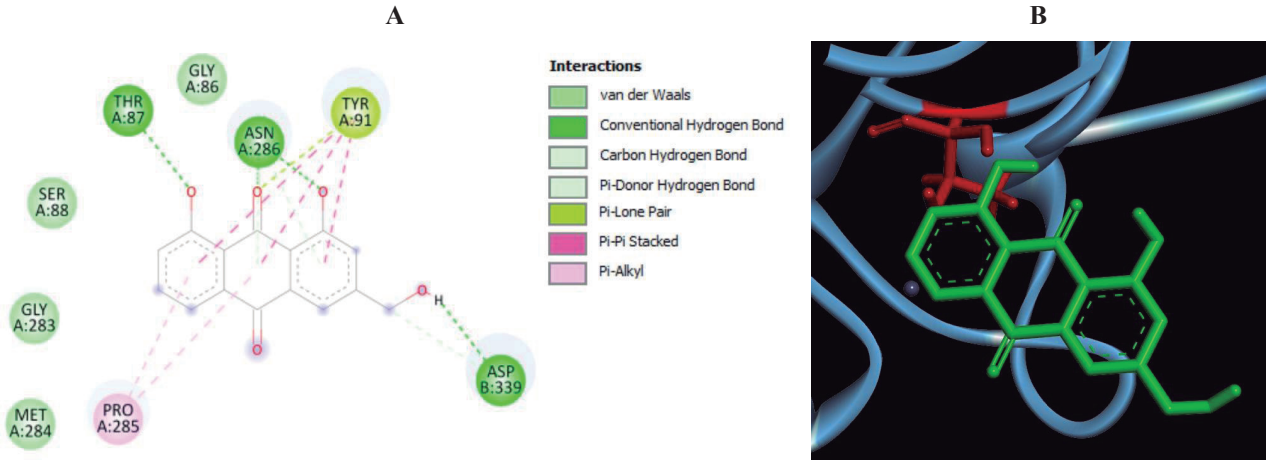
Subsequently, the AutoDock software was configured with the aforementioned data, and the results obtained with AE showed a binding affinity of -6.29 kcal/mol and an interaction efficacy of -0.22 (Table 1), while carvacrol exhibited a binding affinity of -6.06 kcal/mol with a binding efficiency of -0.55 (Table 1).

Table 1 - Results obtained in docking molecular.

Ligant	Binding affinity (kcal/mol)	Interaction efficacy
AE	-6.29	-0.22
Carvacrol	-6.06	-0.55

The next step involved the use of the Biovia Discovery Studio Visualizer software to visualize whether the compounds interacted with protein residues. Since only one residue was observed, a 2D diagram was created for each compound, illustrating their interactions with all amino acids' residues obtained. In figure 1, it is possible to visualize that AE had van der Waals interactions with the residues GLY 86, SER 88, GLY 283, and MET 284, considered one of the weakest. It also presented π covalent bonds with alkyl groups of the PRO 285 residue, which is also a weak interaction. The TYR 91 residue has several interactions with the ligand, such as covalent bonding with a pair of non-shared electrons between a ketone group and covalent bonding with the aromatic rings present in the molecule. This compound also forms hydrogen bond interactions with the residues THR 87, ASN 286, and ASP 339, demonstrating the high affinity of this compound with the enzyme's active site, making it a promising alternative for colistin-based treatments.

Figure 1 - Different representations of the binding between AE and MCR-1 protein responsible for colistin resistant bacteria

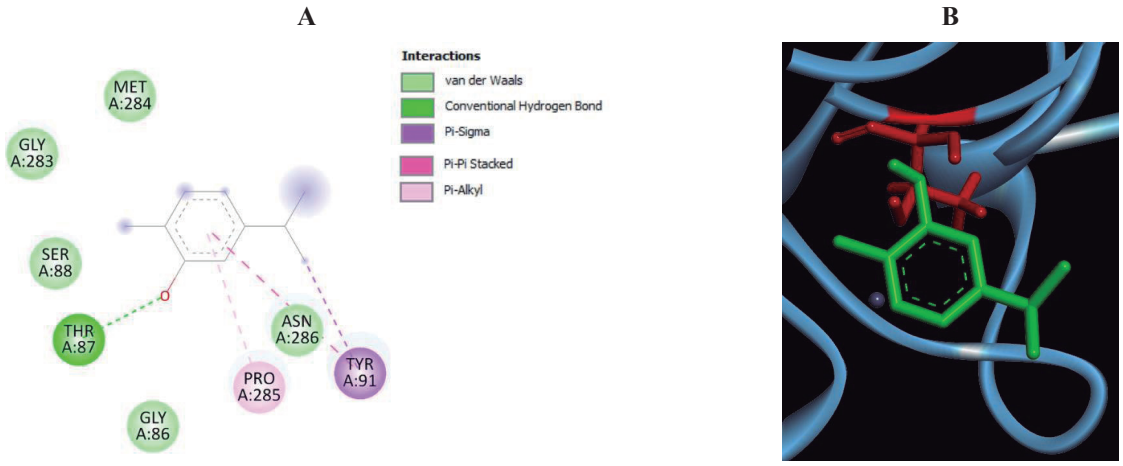


A: Two-dimensional representation of interactions between AE and MCR-1 protein, indicating the type of interaction and the abbreviation for each involved residue. The abbreviations represent ASN: asparagine; ASP: aspartate; GLY: glycine; MET: methionine; PRO: proline; SER: serine; THR: threonine; TYR: tyrosine.
B: Three-dimensional representation of the interaction between AE and MCR-1 protein. BLUE: Part of the MCR1 protein. RED: Active site of the protein, Threonine 87 residue. GREEN: Chemical structure of AE.

With carvacrol in figure 2, there were van der Waals interactions with the residues GLY 86, SER 88, GLY 283, MET 284, and ASN 286. It also exhibited π covalent bonds with alkyl groups of the PRO 285 residue. Additionally, it has a double covalent bond with the TYR 91 residue, which is a stronger type of bond than the π bond. This ligand also forms hydrogen bond interactions with the protein's active site. Analyzing these interactions, it is possible to observe that the protein's active

site has a high affinity for oxygen atoms, indicating a characteristic that should be further investigated in new studies.

Figure 2 - Different representations of the binding between carvacrol and MCR-1 protein.



A: Two-dimensional representation of interactions between carvacrol and MCR-1 protein, indicating the type of interaction and the abbreviation for each involved residue. The abbreviations represent ASN: asparagine; GLY: glycine; MET: methionine; PRO: proline; SER: serine; THR: threonine; TYR: tyrosine.
B: Three-dimensional representation of the interaction between carvacrol and MCR-1 protein. BLUE: Part of the MCR-1 protein. RED: Active site of the protein, Threonine 87 residue. GREEN: Chemical Structure of carvacrol.

The broad-spectrum bacterial resistance approach poses a substantial challenge in the contemporary public health context. In parallel with in vitro experiments, computational simulations serve as a valuable complementary tool, fostering a more comprehensive understanding of underlying enzymatic interactions. The imperative for in-depth investigations regarding phytotherapies as viable alternatives emerges as a pressing requirement. In this context, the need for a collaborative and multidisciplinary effort involving researchers, healthcare professionals, and the pharmaceutical industry becomes evident. This collaboration is essential to effectively address the growing challenge of bacterial resistance and identify innovative solutions that can preserve the effectiveness of antimicrobial treatments. Ongoing research in this area is crucial for ensuring public health and patient safety, emphasizing the importance of exploring the advantages of phytotherapies as an integral part of this strategy.

According to Son and colleagues (2019), it is essential that new antibacterial drugs be developed focusing on the active sites of proteins that enable resistance to certain medications. It was observed that the zinc ion in the MCR-1 protein is crucial for the function of the active site. In the presence of ethylenediaminetetraacetic acid (EDTA), which removed metals from the protein, the resistant activity of the protein was nullified, thereby allowing colistin to take effect. Trindade and colleagues (2023) tested the antibacterial capacity of various natural compounds against the MCR-1 protein, with AE yielding the best results, consistent with this study. The researchers examined curcumin and hypericin, in addition to AE, with binding efficiency results of -6.31 kcal/mol for AE,

-5.86 kcal/mol for curcumin, and -5.72 kcal/mol for hypericin. Given that these natural products are photosensitive, there is a potential for enhanced treatment efficacy. According to Semenyuta and colleagues (2023), they tested derivatives of 1,3-oxazole, a synthetic organic compound, as potential antibacterial against colistin-resistant E. coli, using in silico and in vitro methods. The results obtained showed that types 1 and 3 exhibited the highest antimicrobial activity, with molecular docking yielding binding energy results of -10.1 and -9.5 kcal/mol, respectively. In vitro analysis revealed inhibition halo sizes ranging from 17 to 27 mm. This study supports the current research on the effectiveness of using natural products in combating drug-resistant pathogens. In the study by Ghirga and colleagues (2020), a natural compound was tested to inhibit colistin-resistant bacteria. The enzyme ArnT is present in the cytoplasmic membrane of Pseudomonas aeruginosa bacteria and is responsible for catalyzing the final step of aminoarabinosylation of lipid A. The chosen compound was BBN149, a diterpene isolated from the leaves of Fabiana densa var. ramulosa, known for enhancing the activity of colistin against bacteria. The binding energy result was 5.05 kcal/mol in the in-silico method, and in the in vitro method, BBN149 significantly inhibited the growth of P. aeruginosa in conjunction with colistin at concentrations above 31μM.

In the study by Aldarhami and colleagues (2023), the objective was to investigate the antimicrobial activity of the seed extract of Pithecellobium dulce against pathogenic bacteria through molecular docking. The average binding energy results of the compounds present in the extract with Staphylococcus aureus were -6.8 kcal/mol, indicating the antimicrobial property of this plant. It was also reported that the use of molecular docking is essential for determining a new drug. In Peyclit, Baron, and Rolain's research (2019), the reuse of medications, their characteristics, and the potential for use against antibiotic-resistant bacteria were analyzed. Antibiotic reuse is a more cost-effective alternative compared to developing new drugs; however, the drawbacks of cellular DNA damage and the high inhibitory concentration required are unfavorable aspects of this practice. It was concluded that combining antibiotics can lead to adverse reactions, so new tests with different molecules, whether synthetic or natural, are necessary through computational simulation and laboratory experiments. In Rahman and colleagues' study (2022), new natural antibacterial compounds were investigated for treating infections caused by drug-resistant *Burkholderia cenocepacia*, with the assistance of artificial intelligence. A total of 2335 compounds were tested to predict their antibacterial activity, and only five previously uncharacterized molecules exhibited growth inhibitory activity against *B. cenocepacia*. It was concluded that the use of artificial intelligence in the search for new drugs is an excellent starting point in this research area, as it reduces costs associated with reagents, for example. This study is in agreement with Polito and colleagues (2022), who characterized the essential oil of O. Vulgare ssp. vulgare concerning bacterial isolates, assessing its ability to antagonize the growth of opportunistic human pathogens belonging to the *B. cepacia* complex. The oil was effective, as the percentage of inactivated bacterial cells was approximately 98%.

CONCLUSION

In summary, both AE and O. vulgare proved to be effective antimicrobial agents in scientific studies. Their antibacterial properties have the potential to play a significant role in combating infections caused by pathogenic microorganisms, as these compounds can act as adjuncts in a colistin treatment, while inhibiting the action of the MCR-1 protein, they complement colistin activity, which acts on bacteria cell wall and eliminates them. The increasing research and understanding of these natural substances highlight the importance of exploring their use as alternatives or supplements to conventional antimicrobial treatments. However, it is crucial to continue investigating these substances in different contexts and concentrations to better understand their spectrum of action, therapeutic potential, and possible side effects. It's worth noting that this study was entirely conducted through silico methods. Therefore, additional analyses aimed at elucidating the antimicrobial mechanism of action of AE and carvacrol, as well as in vitro and in vivo effects, can be conducted. With a careful and ongoing approach, AE and O. vulgare may become valuable tools in the fight against infections, contributing to public health and patient well-being.

ACKNOWLEDGEMENT

This work was supported by FAPERGS (Foundation for the Supporting of Research in the State of Rio Grande do Sul) under Grant Number 23/2551-0000776-2.

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