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ANTIMALARIAL POTENTIAL OF NATURAL ROTENOID EXTRACTED FROM PLANT *Clitoria fairchildiana*: A STRATEGY IN MOLECULAR DOCKING

POTENCIAL ANTIMALARIAL DO ROTENÓIDE NATURAL EXTRAÍDO DA PLANTA *Clitoria fairchildiana*: UMA ESTRATÉGIA EM DOCKING MOLECULAR

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Abstract

Malaria is an arbovirosis present in several regions in which febrile illness caused by the Plasmodium parasite is characterized, being considered an acute life-threatening disease and a notable threat to global health. It is noteworthy that the methods of prevention are based on synthetic insecticides which can aggravate symptoms in patients. Thus, the insertion of natural compounds can contribute and improve the development of new ones for arboviroses. In this context, the objective of the study is the development of new therapeutic systems through natural compounds against the etiologic agent that causes malaria, aiming at the popularization of new approaches through the use of natural compounds in the treatment of neglected diseases. The strategy was based on *in silico* model in molecular docking, that is, the formation of an energetic complex between protein (DBL3x domain of the *Plasmodium falciparum* VAR2CSA) with the

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ligands obtained from *C. fairchildiana* (6_deoxyclitoriacetal, 9_demethylclitoriacetal, 11_deoxyclitoriacetal, Cloriacetal, Stemonal, and Stemonone) considering energetic parameters, RMSD (Root Mean Square Deviation) and interactions between the ligands and amino acids of the protein (VAR2CSA). Therefore, the compounds from *C. fairchildiana* presented energy and RMSD values within the suggested parameters, however, all the ligands did not interact with the residues of the active site of the protein. However, we cannot rule out the possibility of inhibition by allosteric modulation of the enzyme. It is emphasized that the study consists of an initial stage for the therapeutic development against neglected diseases using mainly the compounds extracted from *C. fairchildiana* due to its wide dissemination in Brazil and ethnopharmacological potential and expansion *in vitro* and *in vivo* studies for further validation.

Keywords: Virtual Screening; Malaria; Flavonoids; Therapeutic Systems; Public Health.

Resumo

A malária é uma arbovirose presente em diversas regiões em que se caracteriza a doença febril causada pelo parasita Plasmodium, sendo considerada uma doença aguda com risco de vida e uma notável ameaça à saúde global. Vale ressaltar que os métodos de prevenção são baseados em inseticidas sintéticos que podem agravar os sintomas nos pacientes. Assim, a inserção de compostos naturais pode contribuir e melhorar o desenvolvimento de novos para as arboviroses. Neste contexto, o objetivo do estudo é o desenvolvimento de novos sistemas terapêuticos através de compostos naturais contra o agente etiológico causador da malária, visando a popularização de novas abordagens através do uso de compostos naturais no tratamento de doenças negligenciadas. A estratégia foi baseada em modelo *in silico* em docking molecular, ou seja, a formação de um complexo energético entre a proteína (domínio DBL3x do *Plasmodium falciparum* VAR2CSA) com os ligantes obtidos de *C. fairchildiana* (6_deoxyclitoriacetal, 9_demethylclitoriacetal, 11_deoxyclitoriacetal, Cloriacetal, Stemonal e Stemonone) considerando parâmetros energéticos, RMSD (Root Mean Square Deviation) e interações entre os ligantes e os aminoácidos da proteína (VAR2CSA). Portanto, os compostos de *C. fairchildiana* apresentaram valores de energia e RMSD nos parâmetros sugeridos, porém, todos os ligantes não interagiram com os resíduos do sítio ativo da proteína. No entanto, não podemos descartar a possibilidade de inibição por modulação alostérica da enzima. Ressalta-se que o estudo consiste em uma etapa inicial para o desenvolvimento terapêutico contra doenças negligenciadas utilizando principalmente os compostos extraídos de *C. fairchildiana* devido a sua ampla disseminação no Brasil e potencial etnofarmacológico e ampliação dos estudos *in vitro* e *in vivo* para posterior validação.

Palavras-chave: Virtual Screening; Malária; Flavonóides; Sistemas terapêuticos; Saúde pública.

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1. Introduction

Neglected tropical diseases (NTDs) are marked consequences of the underdevelopment that affects important geographical regions of the planet, causing sensitive losses in three vital components: physical, mental, and social health (BEYRER et al., 2007). About one-sixth of the world population, approximately one billion people, are affected by some NTD, including diseases caused by protozoa (Chagas' disease, leishmaniasis, sleeping sickness, malaria), helminths (filariasis, ascariasis, onchocerciasis, schistosomiasis) bacteria (tuberculosis, leprosy, trachoma, Buruli ulcer) and viruses (dengue, yellow fever, rabies) (LIMA-CAMARA et al., 2016).

In this context, malaria is an arboviruses present in several regions (Northeast, Southeast, Midwest, and South) and potentially serious, infectious, and acute characterized by febrile conditions, caused by the *Plasmodium* parasite, in which its transmission usually occurs through the bite of infected *Anopheles* mosquitoes, as well as through the sharing of needles, blood transfusion, or during pregnancy (LOIOLA, SILVA & TAUIL, 2002; TALAPKO et al., 2019).

According to 2018 data from the World Health Organization (WHO), about 228 million cases of malaria have been reported and 405,000 deaths have been reported worldwide, with Africa having the highest number of cases and mortality, meaning that malaria is an acute life-threatening disease and represents a notable threat to global health (WHO, 2019).

It is noteworthy that the preventive measures against malaria were based on the intradomiciliary insertion of dichlorodiphenyltrichloroethane (DDT) against the transmitting anophelines transmitters and the use of antimalarial drugs (chloroquine) to deplete the sources of infection, which over the years has raised great concern about the effectiveness of these therapeutic practices (LOIOLA, SILVA & TAUIL, 2002).

In the interest of biosafety in therapeutic practice, the development of bioactive compounds from plants has been observed, in which the therapeutic properties of many secondary metabolites lead to the exploration and identification of natural products that can lead to the development of new therapeutic substances (DE SÁ-FILHO et al., 2021).

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Given this, computational techniques have helped in the development and optimization of new drugs, because they enable initial trials with greater predictability and reduced the costs of experimental trials (OLIVEIRA, 2020). In this context, molecular docking is a crucial step in drug development, which focuses on the formation of complexes between protein-protein and protein-ligand so that the process of protein inhibition occurs, enabled by the analysis of physicochemical, structural, energetic, reactivity, toxicological properties, among others, promoting initial results of new drugs (DA SILVA; DOS SANTOS NEGREIROS, & DOS SANTOS, 2022).

The genus Clitoria belongs to the Leguminosae family widely distributed in tropical regions and less dispersed in temperate regions. Some species make up the ethnopharmacology such as *C. macrophylla* used for the treatment of skin diseases and commercialized as a raw drug in every market (PITAKPAWASUTTHI et al., 2019). The roots of *C. ternatea* are expectorant agents in the treatment of sore throats and lung infections (KUMAR & DHOBI, 2018). And *C. fairchildiana*, popularly known as "sombrero", is located in several regions of the Brazilian territory and is very suitable for cultivation as a shade tree, but there are still few reports of medicinal use in neglected diseases (BERTONCELI al., 2022).

Thus, this study aims to develop new therapeutic systems through computational technique (molecular docking) between natural rotenoids extracted from the plant *C. fairchildiana* against malaria, which is a disease that establishes great concern in health sectors due to its epidemiology, occurrence and chain of transmission. Aiming at the popularization of new studies of innovative character in the search for biologically active molecules for the treatment of neglected diseases.

2. Literature review

2.1 The use of molecular docking in drug development

Drug development is an extensive and costly procedure, which can require a period of close to 8 years and an estimated investment of 985 million dollars to identify, evaluate, and make the drug available on the market. Computational

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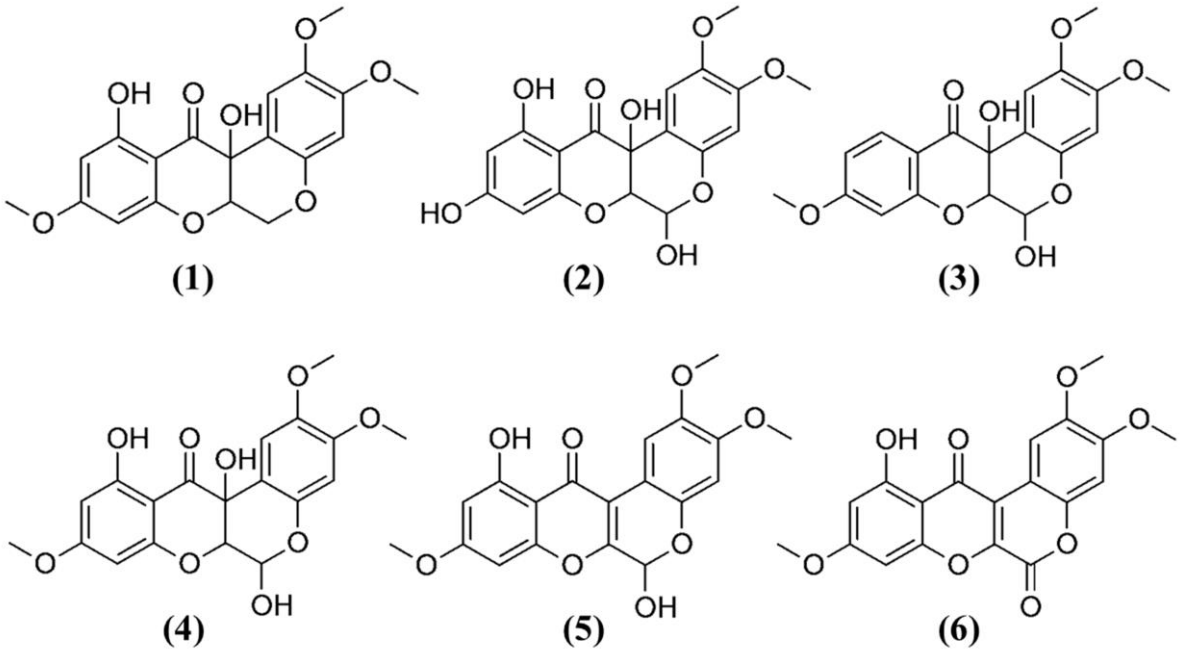
methods have therefore proved to be an efficient way of developing drugs, as they considerably reduce financial costs and time (DU et al., 2023).

The molecular docking technique is used to assess the inhibition of a protein through interactions with the ligand. The procedure is represented in 3D and the enzyme is acquired using X-ray crystallography, nuclear magnetic resonance spectroscopy, or cryoelectron microscopy (AGU et al., 2023). The evaluation is based on electrostatics and thermodynamics, to quantitatively indicate the affinity of the bond and its most favorable position, indicating the pharmacological activity of the ligand (SUMARYADA & PRAMUDITA, 2021).

3. Methodology

The natural rotenoids extracted from the plant C. fairchildiana evaluated were: (6_deoxyclitoriacetal (1), 9_demethylclitoriacetal (2), 11_deoxyclitoriacetal (3), Cloriacetal (4), Stemonal (5) and Stemonone (6)) (Figure 1) against malaria characteristic protein (SANTOS, DAVID & DAVID, 2016; MATHIAS, MORS & PARENTE, 1998; MATHIAS et al., 2005).

Figure 1. Two-dimensional structures of *C. fairchildiana* extracted



Source: Adapted from SANTOS, DAVID & DAVID, 2016; MATHIAS, MORS & PARENTE, 1998; MATHIAS et al., 2005.

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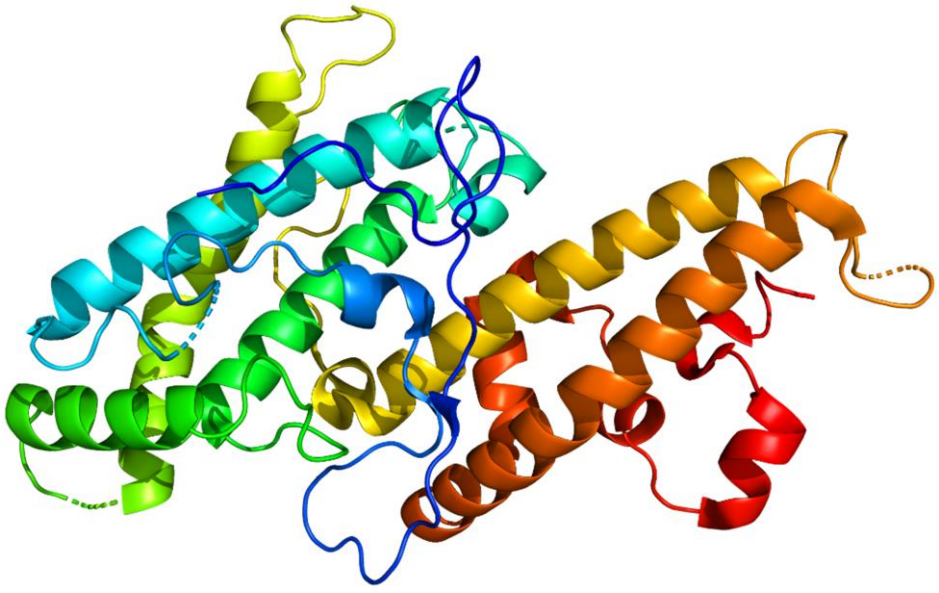
In literary context, Reges et al. (2019); De Oliveira et al. (2020); Lucio et al. (2019); De Oliveira et al. (2019) established through computational calculations data of the boundary orbitals of the compounds cloriacetal, 6-deoxycloriacetal, stemonone and stemonal scoring the following parameters: HOMO and LUMO boundary orbitals, GAP, reactivity descriptors, dipole moment and Mulliken charges from which they proposed initial chemical investigation studies of these compounds.

All ligands went through a structural and geometric optimization process through the parameters established by the classical force field method (MMFF94 - Merck Molecular Force Field 94), aiming to obtain the lowest value of potential energy and thus deduce its most stable three-dimensional structure, such process was performed by the software Avogadro® (HALGREN, 1996; HANWELL et al., 2012).

Obtaining the target in .pdb format, occurred through the Protein Data Bank virtual repository (https://www.rcsb.org/), with the identification code (PBD ID: 3CML, Crystal Structure of the DBL3x domain of the *Plasmodium falciparum* VAR2CSA protein) (Figure 2), However, the three-dimensional structure has only water molecule residues in its composition, and also has the following amino acids (Lys1324, Arg1467, Arg1503, Lys1504, Lys1507, and Lys1510) present in the active site of the protein (SPADETO et al., 2021; SINGH et al., 2008).

Figure 2. Structure of the DBL3x domain of the *Plasmodium falciparum* VAR2CSA protein



Source: Authors

For the progress of the molecular docking protocol were removed all residual structures that could cause in the direct interference in the formation of the complex between the proteins with the ligands, the removal of residues was given through the chimera® software, then the protein files were directed to the autodocktools® software for conversion into .pdbqt format and incorporate the gridbox calculation for the protein (MORRIS et al., 2009; PETTERSEN et al., 2004).

The gridbox calculation helps in the delimitation of the acting region in which the ligands will have during the simulation, the gridbox was denoted around the whole protein region making possible to open margin for a bigger interaction for the protein (*Plasmodium falciparum* VAR2CSA protein) the gridbox parameters were center X: -8.651, center Y: -9.877, center Z: 27.552, X-dimension: 78, Y-dimension: 76 and Z-dimension: 126 with spacing of 0.647Å.

In short, all molecular docking simulations are in silico using the software autodockvina®, to obtain the calculations of complex formation between protein-ligand for each ligand and protein were developed 100 simulations with 20 possibilities of interactions that followed evaluations through data provided at the end of each simulation (GAILLARD, 2018).

At the end of the simulation, as criteria are studied the results of RMSD - Root Mean Square Deviation with values in angron scale and the free energy of binding (ΔG), for both parameters the lower its value the better it will be for the formation of the complex, thus, it is suggested to use values less than 2.0 for RMSD and results equal to or less than -6.0 kcal/mol for the binding energy (SHITYAKOV & FÖRSTER, 2014).

The three-dimensional figures of the proteins in complex with each ligand evaluated in the research were produced with the help of Discovery Studio Visualize® and Chimera®, and the acquisition and identification of the interactions were performed with the help of the Protein-Ligand Interaction Profiler website (https://acesse.dev/plilp-tool) (BIOVIA, 2000; PETTERSEN et al., 2004).

The results from the simulations in which the complexes formed with the protein (*Plasmodium falciparum* VAR2CSA protein), and each ligand have a specific value regarding the free energy of binding and RMSD resulting from each complex. Thus, each ligand incorporates a direct interaction with the target protein, consequently interfering in its interactions with the amino acids present in the

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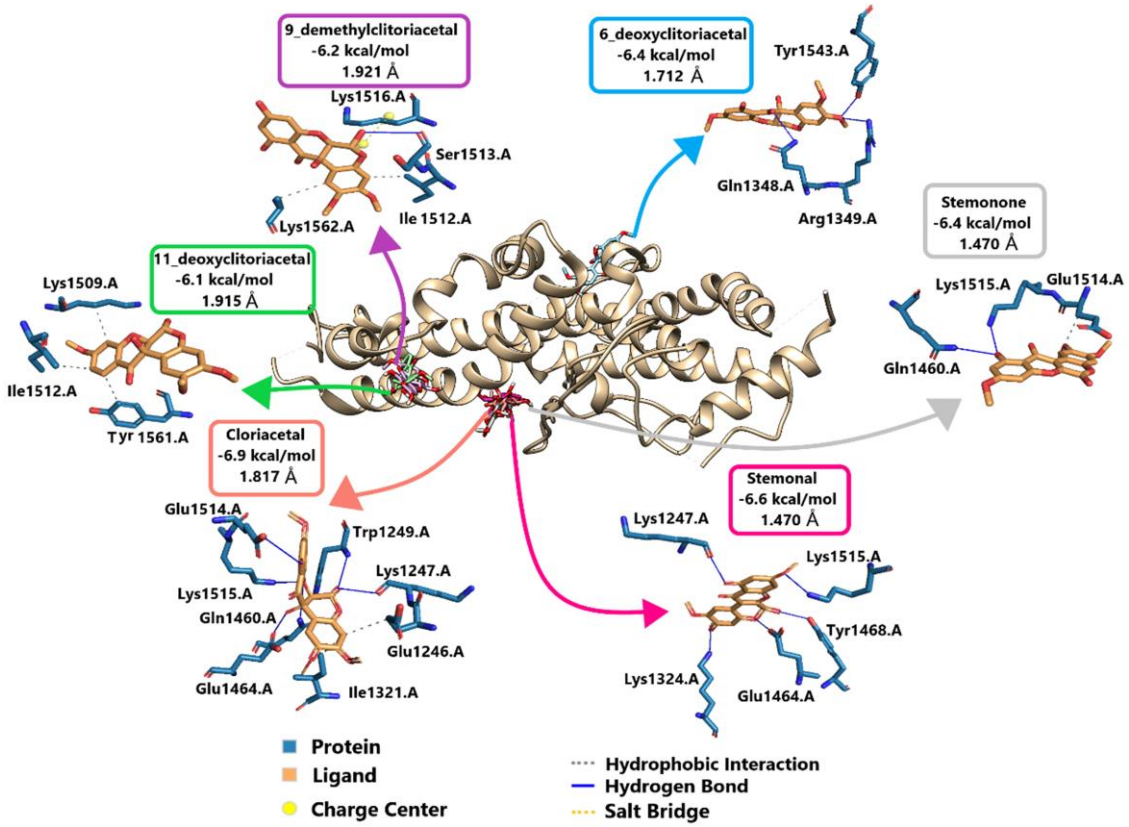
protein.

4. Results and Discussion

Initially, delineates divergences in protein-ligand complex formation, energy values, and RMSD. In summary, the ligands cloriacetal, stemonal and stemonone showed similarity in a complexed region, but the ligands 9_demethylchlitoriacetal, 11_deoxyclitoriacetal also complexed in a region common to each other, and the ligand 6_deoxyclitoriacetal a region that differs from the others (Figure 3).

Aiming at the specificities of each complex, it is noted that the ligand 6-deoxycyclitoriacetal, showed divergence in its binding, because of the location where it made the formation of its complex, having an energy value equal to -6.4kcal/mol and RMSD of 1.712 Å, interacted with three amino acids Tyr1543.A, Gln1348.A and Arg1349.A, however, none belongs to the active site.

Figure 3. Complexes formed by the ligands with the VAR2CSA protein



Source: Authors

Among the ligands, 9_demethylclitoriacetal and 11_deoxyclitoriacetal that

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showed similarity for the formation of the complex in the three-dimensional structure of the protein the 9_demethylclitoriacetal has -6.2 kcal/mol energy and 1.921Å and his interactions with the amino acids Lys1516.A, Ser1513.A, Ile1512. The Lys1562.A with compound 11_deoxyclitoriacetal has an energy value equal to -6.1 kcal/mol and the RMSD 1.915Å and has three interactions with the amino acids Lys1509.A, Ile1512.A and Tyr1561.A, of all the exerted interactions no ligand has a binding with any amino acid present in the active site.

The ligands Cloriacetal, Stemonal, and Stemonone showed the same behavior when complexing in the protein structure, in which Cloriacetal has -6.9 kcal/mol as energy value and its RMSD is equivalent to 1. 817Å and interacted with the amino acids (Glu1514.A, Lys1515.A, Gln1460.A, Glu1464.A, Ile1321.A, Glu1246.A, Lys1247.A, and Trp1249.A); while Stemonal has energy equivalent to -6.6 kcal/mol and its RMSD is equivalent to 1. 470Å, Stemonal exerted five interactions with the amino acids Lys1247.A, Lys1324.A, Glu1464.A, Tyr1468.A and Lys1515.A, the compound Stemonone has an energy value equal to -6.4 kcal/mol and its RMSD is equivalent to 1. 470Å, and about its interactions is dated the amino acids Lys1515.A, Glu1514.A and Gln1460.A, but concerning all interactions are pointed out that the analyzed amino acids do not integrate the interaction site of the active site of the protein.

Accordingly, the values of distances that each ligand interacted to the amino acids of the protein are highlighted in (Table 1). Pointed out all the interactions that the 6_deoxyclitoriacetal ligand, correlated the type of each interaction with the values of the distances, in which it exerted three hydrogen bonds with residues Gln1348.A - 2.68Å, Arg1349.A - 2.78Å, Tyr1543.A - 1.98Å, where the best distance was realized by the amino acid Tyr1543.A.

The 9_demethylclitoriacetal ligand present in Table 1, showed four bonds where two are hydrophobic bonds with residues Ile1512.A - 3.42Å, Lys1562.A - 3.81Å; one hydrogen bond with residue Ser1513.A - 2.84Å, and only one Salt Bridge with residue Lys1516.A at 4.61 Å. The interactions of the ligand 11_deoxyclitoriacetal, are bounded in three hydrophobic bonds with residues Lys1509.A, Ile1512.A and Tyr1561.A, their distances being equivalent to 3.61Å, 3.54Å and 3.09Å, respectively.

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Table 1. Distance values of the ligands studied with the VAR2CSA protein

Compounds	Energy (kcal/mol)	RMSD (Å)	Interactions	Bond type	Distance (Å)
6_deoxyclitoriacetal	-6.4	1.712	Gln1348.A	H-Bond	2.68
			Arg1349.A	H-Bond	2.78
			Tyr1543.A	H-Bond	1.98
9_demethylclitoriacetal	-6.2	1.921	Ile1512.A	Hydrophobic	3.42
			Lys1562.A	Hydrophobic	3.81
			Ser1513.A	H-Bond	2.84
			Lys1516.A	Salt Bridges	4.61
11_deoxyclitoriacetal	-6.1	1.915	Lys1509.A	Hydrophobic	3.61
			Ile1512.A	Hydrophobic	3.54
			Tyr1516.A	Hydrophobic	3.09
Cloriacetal	-6.9	1.817	Glu1246.A	Hydrophobic	3.84
			Ile1321.A	Hydrophobic	3.68
			Lys1247.A	H-Bond	2.18
			Trp1249.A	H-Bond	2.81
			Gln1460.A	H-Bond	3.30
			Glu1464.A	H-Bond	2.04
			Glu1514.A	H-Bond	3.11
			Lys1515.A	H-Bond	2.61
Stemonal	-6.6	1.507	Lys1247.A	H-Bond	2.17
			Lys1324.A	H-Bond	3.21
			Glu1468.A	H-Bond	3.16
			Tyr1268.A	H-Bond	2.45
			Lys1515.A	H-Bond	2.37
Stemonone	-6.4	1.470	Glu1514.A	Hydrophobic	3.76
			Gln1460.A	H-Bond	2.62
			Lys1515.A	H-Bond	2.16

Source: Authors

The compost cloriacetal constituted two hydrophobic interactions with residues Glu1246.A and 1321.A with the results of distances 3.84Å and 3.68Å, respectively; hydrogen bonds were performed with six residues the Lys1247.A, Trp1249.A, Gln1460.A, Glu1464.A, Glu1514.A and Lys1515.A, the shortest distance being 2.18, with residue Lys1247.A.

The Stemonal ligand, it was contacted in table 1, all its interactions performed with some amino acids present in the protein, the residues are:

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Lys1247.A, Lys1324.A, Glu1468.A, Tyr1268.A and Lys1515.A, being the best distance exerted by the amino acid Lys1247.A with the result 2.17Å.And lastly, we have the Stemonone ligand that interacted with only three amino acids inserted in the protein where one of them has a hydrophobic character with Glu1514.A with its distance of 3.76; in relation to hydrogen bonding, we have residues Gln1460.A and Lys1515.A, with their respective distances of 2.62Å and 2.16Å.

5. Conclusion

In summary, the malaria characteristic protein (VAR2CSA), possessed the site of interest of Lys1324, Arg1467, Arg1503, Lys1504, Lys1507 and Lys1510, the ligands (6_deoxyclitoriacetal, 9_demethylclitoriacetal, 11_deoxyclitoriacetal, Cloriacetal, Stemonal and Stemonone) through computer simulation in which pointed out that all ligands have energy values and RMSD within the suggested parameters and that all ligands do not interact with the residues of the active site of the protein, thus, it is deduced that the ligands (6_deoxyclitoriacetal, 9_demethylclitoriacetal, 11_deoxyclitoriacetal, Cloriacetal, Stemonal and Stemonone), However, we cannot rule out the possibility of inhibition via allosteric modulation which occurs when the ligands interact with amino acid residues, which do not make up the active site of the enzyme but modify the three-dimensional structure of the enzyme causing inhibition. It is worth mentioning the importance of the results obtained through computer simulations and theoretical studies in the screening of new drugs. However, it is emphasized that new *in vitro* and *in vivo* studies must be carried out for further validation of the results the action of the molecules described here against the etiological agent of malaria.

References

AGU, P. C. et al. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. **Scientific Reports**, v. 13, n. 1, p. 1-18, 2023. Available at: https://www.nature.com/articles/s41598-023-40160-2. Accessed on: June 12, 2024.

BERTONCELI, Maria Aparecida Aride et al. Rotenoids from Clitoria fairchildiana R. Howard (Fabaceae) seeds affect the cellular metabolism of larvae of Aedes aegypti L.(Culicidae). **Pesticide Biochemistry and Physiology**, v. 186, p. 105167, 2022. Available at: https://encurtador.com.br/Rss68. Accessed on: June 12, 2024.

BEYRER, Chris et al. Neglected diseases, civil conflicts, and the right to health. **The Lancet**, v. 370, n. 9587, p. 619-627, 2007. Available at: https://encurtador.com.br/MoY8W. Accessed on: June 12, 2024.

BIOVIA, Dassault Systemes et al. Dassault systèmes BIOVIA, discovery studio visualizer, v. 17.2, San Diego: Dassault Systèmes, 2016. **J Chem Phys**, v. 10, p. 21-9991, 2000. Available at: https://discover.3ds.com/discovery-studio-visualizer-download. Accessed on: June 12, 2024.

DA SILVA, Maria Rosana Marques; DOS SANTOS NEGREIROS, Polyanna; DOS SANTOS, Pauline Sousa. Integração do uso de técnicas de análise computacional na P&D farmacêutica moderna. **Research, Society and Development**, v. 11, n. 7, p. 1-11, 2022. Available at: https://doi.org/10.33448/rsd-v11i7.29672. Accessed on: June 12, 2024.

DE OLIVEIRA, David Lopes Santiago et al. Characterization of the natural pesticide 6-desoxyclitoriacetal: A quantum study. **International Journal of Recent Research and Review**, v. 10, p. 1149-1156, 2019. Available at: https://encurtador.com.br/oJU3U. Accessed on: June 12, 2024.

DE OLIVEIRA, Victor Moreira et al. Characterization of rotenoid stemonal by semiempirical methods and molecular docking. **SN Applied Sciences**, v. 2, n. 4, p. 699, 2020. Available at: https://doi.org/10.1007/s42452-020-2346-7. Accessed on: June 14, 2024.

DE SÁ-FILHO, Geovan Figueirêdo et al. Medicinal plants used in the Brazilian caatinga and the therapeutic potential of secondary metabolites: a review. **Research, Society and Development**, v. 10, n. 13, 2021. Available at: https://rsdjournal.org/index.php/rsd/article/view/21096. Accessed on: June 14, 2024.

DU, Lianming et al. Dockey: a modern integrated tool for large-scale molecular docking and virtual screening. **Briefings in Bioinformatics**, v. 24, n. 2, p. 1-7, 2023. Available at: https://encurtador.com.br/O6GfL. Accessed on: June 14, 2024.

GAILLARD, Thomas. Evaluation of AutoDock and AutoDock Vina on the CASF-2013 benchmark. **Journal of chemical information and modeling**, v. 58, n. 8, p. 1697-1706, 2018. Available at: https://doi.org/10.1021/acs.jcim.8b00312. Accessed on: June 14, 2024.

HALGREN, Thomas A. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. **Journal of computational chemistry**, v. 17, n. 5-6, p. 490-519, 1996. Available at: https://encurtador.com.br/3g61D. Accessed on: June 14, 2024.

HANWELL, Marcus D. et al. Avogadro: an advanced semantic chemical editor, visualization, and

analysis platform. **Journal of cheminformatics**, v. 4, p. 1-17, 2012. Available at: https://doi.org/10.1186/1758-2946-4-17. Accessed on: June 14, 2024.

KUMAR, D.; DHOBI, M. Screening antianxiety and antioxidant profile of stems and leaves of blue variety of clitoria ternatea L. **Indian Journal of Pharmaceutical Sciences**, v. 79, n. 6, p. 1022, 2017. Available at: https://doi.org/10.4172/pharmaceutical-sciences.1000322. Accessed on: June 14, 2024.

LIMA-CAMARA, Tamara Nunes. Arboviroses emergentes e novos desafios para a saúde pública no Brasil. **Revista de Saúde Pública**, v. 50, p. 36, 2016. Available at: https://doi.org/10.1590/S1518-8787.2016050006791. Accessed on: June 14, 2024.

LOIOLA, Carlos Catão Prates; SILVA, C. J.; TAUIL, Pedro Luiz. Controle da malária no Brasil: 1965 a 2001. **Revista Panamericana de Salud Pública**, v. 11, p. 235-244, 2002. Available at: https://www.scielosp.org/pdf/rpsp/v11n4/10469.pdf. Accessed on: June 15, 2024.

LUCIO, Francisco Nithael Melo et al. Semi-Empirical Study Of Rotenone Stemonone: HOMO, LUMO and Reactivity Descriptors. **Int. J. Sci. Eng. Res**, v. 10, n. 4, p. 1332-1336, 2019. Available at: https://acesse.dev/k9NU4. Accessed on: June 15, 2024.

MATHIAS, Leda et al. Isolation and structural elucidation of a novel rotenoid from the seeds of Clitoria fairchildiana. **Natural Product Research**, v. 19, n. 4, p. 325-329, 2005. Available at: https://doi.org/10.1080/14786410412331272022. Accessed on: June 15, 2024.

MATHIAS, Leda; MORS, Walter B.; PARENTE, José P. Rotenoids from seeds of Clitoria fairchildiana. **Phytochemistry**, v. 48, n. 8, p. 1449-1451, 1998. Available at: https://encr.pw/SfiuZ. Accessed on: June 15, 2024.

MORRIS, Garrett M. et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. **Journal of computational chemistry**, v. 30, n. 16, p. 2785-2791, 2009. Available at: https://doi.org/10.1002/jcc.21256. Accessed on: June 15, 2024.

OLIVEIRA, Ana. Como criar um novo fármaco. **Revista de Ciência Elementar**, v. 8, n. 2, 2020. Available at: http://doi.org/10.24927/rce2020.020. Accessed on: June 16, 2024.

PETTERSEN, Eric F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. **Journal of computational chemistry**, v. 25, n. 13, p. 1605-1612, 2004. Available at: https://doi.org/10.1002/jcc.20084. Accessed on: June 16, 2024.

PITAKPAWASUTTHI, Yamon et al. Quality evaluation with reference to clitoriacetal and in vitro antioxidant activities of Clitoria macrophylla root. **Journal of Advanced Pharmaceutical**

Technology & Research, v. 10, n. 4, p. 169-177, 2019. Available at: https://doi.org/10.4103/japtr.JAPTR_67_19. Accessed on: June 16, 2024.

REGES, Miquéias; MARINHO, Emanuelle Machado; MARINHO, Márcia Machado. Theoretical study of the natural insecticide rotenone clitoriacetal. **International Journal of Recent Research and Review**, v. 12, p. 1-5, 2019. Available at: https://encr.pw/UvQBH. Accessed on: June 16, 2024.

SANTOS, Rauldenis AF; DAVID, Jorge M.; DAVID, Juceni P. Detection and quantification of rotenoids from Clitoria fairchildiana and its lipids profile. **Natural product communications**, v. 11, n. 5, p. 1934578X1601100519, 2016. Available at: https://doi.org/10.1177/1934578X1601100519. Accessed on: June 18, 2024.

SHITYAKOV, Sergey; FÖRSTER, Carola. In silico predictive model to determine vector-mediated transport properties for the blood–brain barrier choline transporter. **Advances and Applications in Bioinformatics and Chemistry**, p. 23-36, 2014. Available at: https://www.tandfonline.com/doi/full/10.2147/AABC.S63749. Accessed on: June 18, 2024.

SINGH, Kavita et al. Structure of the DBL3x domain of pregnancy-associated malaria protein VAR2CSA complexed with chondroitin sulfate A. **Nature structural & molecular biology**, v. 15, n. 9, p. 932-938, 2008. Available at: https://doi.org/10.1038/nsmb.1479. Accessed on: June 18, 2024.

SPADETO, Joao P. M. et al. Docking and molecular dynamics studies of potential new leads against DBL3x derived from chondroitin sulfate A (CSA): a new approach for the treatment of malaria. **Journal of Biomolecular Structure and Dynamics**, v. 40, n. 18, p. 8384-8393, 2022. Available at: https://doi.org/10.1080/07391102.2021.1911859. Accessed on: June 18, 2024.

SUMARYADA, Tony; PRAMUDITA, Cindy A. Molecular docking evaluation of some Indonesian's popular herbals for a possible COVID-19 treatment. **Biointerface Res. Appl. Chem**, v. 11, n. 3, p. 9827-9835, 2021. Available at: https://l1nq.com/HWInS. Accessed on: June 18, 2024.

TALAPKO, Jasminka et al. Malaria: the past and the present. **Microorganisms**, v. 7, n. 6, p. 179, 2019. Available at: https://doi.org/10.3390/microorganisms7060179. Accessed on: June 18, 2024.

WHO. **World malaria report 2019**. Geneva: World Health Organization; 2019. Available at: https://www.who.int/publications/i/item/9789241565721. Accessed on: June 18, 2024.