Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v46i1.69364

In silico evaluation of rotenoids extracted from *Clitoria fairchildiana* against the *Aedes aegypti* virus

Damião Sampaio de Sousa*, Anthony Barbosa Belarmino, Victor Moreira de Oliveira, Francisco Rogênio da Silva Mendes, Márcia Machado Marinho and Gabrielle Silva Marinho

Universidade Estadual do Ceará, Rua Américo Vespúcio, 60741-145, Fortaleza, Ceará, Brazil. *Author for correspondence. E-mail: damiao.sampaio1@gmail.com

ABSTRACT. Dengue is an arbovirus that has become a serious public health problem in Brazil, as well as in other tropical regions of the world due to population, economic, political, and social factors that contribute to the proliferation, circulation, and introduction of viral strains in its vectors, the mosquitoes of the genus Aedes. Thus, the objective of this work is to identify molecules extracted from the plant *Clitoria fairchildiana* that are potentially active against dengue viruses (DENV) enabling the development of a new therapeutic system made possible through an *in silico* model. The methodological strategy was based on molecular docking that aims the prediction of interaction characteristics between chemical compounds (ligands) and their macromolecular targets when the structures of both (ligand and receptor) considering as validation parameters: calculations of RMSD - Root Mean Square Deviation, the free energy of binding and biochemical interactions formed between the complexes. From this, among the compounds evaluated in this study, it can be inferred that all compounds have inhibitory potential against dengue protein (NS5MTaseDV), especially compounds 9-demethylclitoriacetal and 11-deoxyclitoriacetal, presenting in the analyses two interactions with the protein reference residues. The present study plays an initial character in the screening of new antiviral compounds and the expansion *in vitro* and *in vivo* research to effect these compounds.

Keywords: Dengue; molecular docking; neglected diseases; natural compounds; public health.

Received on August 21, 2023
Accepted on December 01, 2023

Introduction

Dengue is the most important human arbovirosis in terms of morbidity and mortality (Oliveira, Araújo, & Cavalcanti, 2018; Lowe et al., 2018). Among the re-emerging diseases, it is the most serious public health problem, especially in tropical and subtropical regions, where environmental, political, and social conditions favor the proliferation, circulation, and introduction of viral strains of its vectors, the mosquitoes of the genus Aedes (Wilder-Smith, Ooi, Horstick, & Wills, 2019; Gubler, 2019).

The etiologic agent of dengue is the DENV virus, which belongs to the family Flaviviridae and the genus *Flavivirus* and consists of four closely related but antigenically distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). Dengue viruses (DENV) have a genome consisting of sense-positive RNA expressing structural and nonstructural proteins among which NS1 which plays an activity in viral genome replication and may play a role in modulating cellular signaling pathways (Carneiro et al., 2015).

Dengue constitutes a broad clinical diagnosis, ranging from asymptomatic or oligosymptomatic forms to severe and lethal forms (Zara, Santos, Fernandes-Oliveira, Carvalho, & Coelho, 2016; Terra, Da Silva, Pereira, & Lima, 2017). It is emphasized that the causes of the occurrence of severe forms are still unknown, there are some explanatory theories related to the greater virulence of the infecting virus strain, the sequence of infections by different serotypes of the etiologic agent, individual host factors, and a combination of all previous explanations (Barroso et al., 2020). Because of this, an effective preventive vaccine is not available, thus effective etiologic therapy and chemoprophylaxis cannot yet be counted on. At the moment, the only vulnerable link in the dengue transmission chain to a preventive measure is the vector (Harapan et al., 2020; Harapan, Michie, Sasmono, & Imrie, 2021).

In addition, this arbovirosis is one of the infections of interest in studies due to its relevant economic impact on public health. The average expenditure per individual was estimated at US$ 514 for outpatients and US$ 1394 for hospitalized patients in several countries, including Brazil (Araújo, Bezerra, Amâncio, Passos,

& Carneiro, 2017). This estimate, made by Furuya-Kanamori et al. (2016), does not include spending on epidemiological surveillance and vector control, which would further increase the cost in most cases, the infection is self-limited and lasts about 14 days. However, studies show persistent symptoms in the long term which increases the economic and quality of life losses of patients (Hotez et al., 2014; Pescarini et al., 2022).

In this context, bioinformatics tools for building, simulating, and analyzing 3D structures become fundamental. Virtual screening simulations are typically used early in the drug design process, to test a set of compounds with the potential to show activity against the chosen target. Virtual screening has become an important methodology to precede in vitro assays (Maia, Assis, De Oliveira, Da Silva, & Taranto, 2020a). The virtual screening approach contributes greatly to the drug development process because compounds with the potential to interact with the studied ligand site can be further investigated with greater precision, drastically reducing the prototype identification time when compared to conventional strategies (Lima et al., 2016).

The strategy for approaching structure-based screening is to use a molecular docking program to determine the binding mode of a compound on the protein target from a database of compounds from which it is selected (Maia, Medaglia, Da Silva, & Taranto, 2020b). The conformations obtained are used to approximate the binding free energy or related to the affinity of the compound (Codding, 2013).

The development strategy called molecular docking aims to predict the characteristics of the interaction between chemical compounds (ligands) and their macromolecular targets, when the structures of both (informally called ligand and receptor) are already known experimentally (Piccirillo & Amaral, 2018). There are important reasons to undertake the docking study. One is that it is usually not known which of the possible conformations of the ligand interacts best with the receptor. Moreover, for the molecules of interest in the present project, there is still a need for a global exploration (blind docking) because information about the likely binding site is not yet available (Jakhar, Dangi, Khichi, & Chhillar, 2020).

Another reason is that the differences in toxicological profile and clinical activity spectrum between different compounds are still being studied, indicating that they are related to variations between the analogs in their clinical and molecular pharmacology (Fokoue, Pinheiro, Fraga, & Sant'Anna, 2020; Medeiros Filho, Santos Nascimento, Santos, & Frazão, 2020).

This computational approach allows in silico screening of large libraries of compounds, assessing affinity and specificity from structural and chemical properties such as size, geometry, charge distribution, polarity, and potential for hydrophobic interactions and hydrogen bonds (Santos, Daniel, Próspero, & Costa, 2018).

Thus, the objective of the study was to identify possible natural ligands extracted from the plant *Clitoria fairchildiana* with antiviral activity, aiming at the development of new therapeutic agents through in silico screening for arboviruses transmitted by *Aedes aegypti*.

Methodology

Ligand and receptor preparation for Docking simulations

The compounds selected for the present virtual screening were: 6_deoxyclitoriacetal (1), 9_demethylclitoriacetal (2), 11_deoxyclitoriacetal (3), Cloriacetal (4), Stemonal (5), and Stemonone (6) (Figure 1), all extracted from seeds and roots of *Clitoria fairchildiana* and widely distributed in the literature (Santos, David & David, 2016; Mathias, Mors and Parente, 1998; Mathias, Da Silva, Mors, & Parente, 2005; Pitakpawasutthi, Suwatronnakorn, Issaravanich, Palanuvej, & Ruangrungsi, 2019; Pereira da Silva & Paz Parente, 2002).

C. fairchildiana is a plant made up of compound, alternate, and pinnate leaves that exhibit an intense green hue, which can be found in native areas of the Amazon region (Costa, Silva, & Gomes, 2014; Alves, Alves & Santos-Moura, 2015). Due to its diversity, this species contains a variety of bioactive compounds such as rotenoids (mostly), flavonoids, and alkaloids that have demonstrated antiviral (Bertonceli et al., 2022), antioxidant (Annegowda, Bhat, Tze, Karim, & Mansor, 2013) and anti-inflammatory (Konozy, Osman, & Dirar, 2022) activity, standing out as promising phytochemicals.

Rotenoids are chemical compounds consisting of a fused ring system, often represented by four aromatic rings. This structure gives rotenoids stability and rigidity, characteristics that can influence their interactions with biological targets (Dewick, 2017). Also in his studies, Dewick (2017) describes that the presence of functional groups, such as hydroxyls and methoxyls, in specific positions in the molecular structure contributes to the diversity of these compounds and can influence their biological activities.

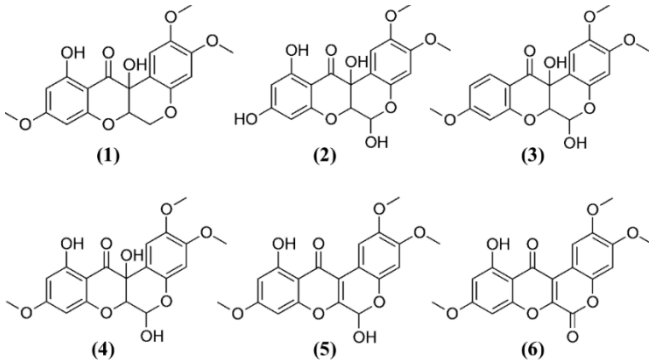

Figure 1. Two-dimensional structures of *C. fairchildiana* extracted.

It should be noted that during the protocol all the ligands were subjected to structural and geometric optimization methods established by the classical Merck Molecular Force Field 94 (MMFF94) method fostered using Avogadro® software to obtain the lowest potential energy value aiming at the most stable three-dimensional structure (Halgren, 1996; Hanwell et al., 2012).

In addition, the studied protein Dengue transferase (NS5MTaseDV) has residues in its three-dimensional structure (Egloff, Benarroch, Selisko, Romette, & Canard, 2002). Thus, incorporating possible direct interferences in the formation of the complex between the protein-ligands, all residual structures were removed through the Chimera® software, then the protein file was directed to the AutodockTools® software for conversion into .pdbtqt format and perform the gridbox calculation (Gaillard, 2018).

The gridbox calculation allows the delimitation of the performance in which the ligands may play during the simulation, i.e., the gridbox allows greater possibilities of interactions between the protein (Dengue transferase NS5MTaseDV) and the ligands. Furthermore, the gridbox parameters correspond to: center X: 8.191, center Y: -46.9, center Z: 1.544, X-dimension: 92, Y-dimension: 110, and Z-dimension: 126 and its spacing equals 0.481.

Molecular docking simulation and Data output/Validation of the docking method

The crystal structure of Dengue transferase NS5MTaseDV was obtained from the Protein Data Bank© web server (https://www.rcsb.org/) with PDB code ID: 1L9K. In addition, it establishes the method: x-ray diffraction; resolution: 2.40Å; R-value free: 0.257 0.257; R-value work: 0.231 and R-value observed: 0.275.

Dengue transferase protein (NS5MTaseDV) presents in its constitution the NS5 RNA-dependent RNA polymerase of flaviviruses with the characteristic character of methyltransferases dependent on S-adenosyl-L-methionine at its N-terminus and polymerase motifs at its C-terminus in which biochemical studies of these protein domains may provide a structural basis for rational drug design against emerging flaviviruses (Egloff et al., 2002).

Furthermore, the NS5MTaseDV protein (green color) has complexed in its three-dimensional structure the ligand S-Adenosyl-L-Homocysteine (SAH) (yellow color) (Figure 2). According to the literature, this ligand interacts with the residues (Val132, Asp131, Lys105 Thr104, Ser56, and Gly86), in which it is pointed out that these amino acids integrate the active site region of the protein (Egloff et al., 2002).

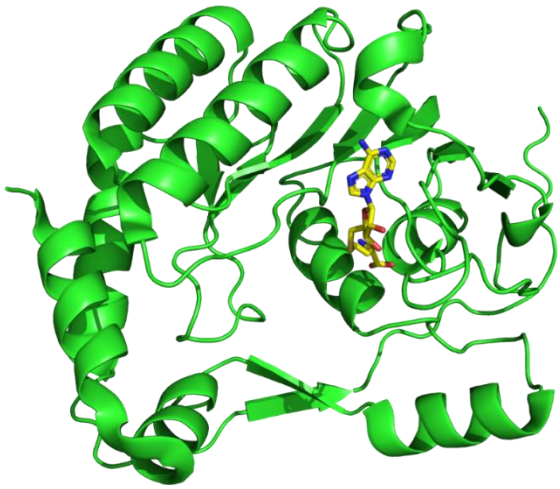

Figure 2. Three-dimensional structure of the NS5MTaseDV protein.

All simulations are *in silico* (molecular docking) given with the use of AutodockVina® software for calculation of complex formation between protein-ligand, for each ligand and protein was performed 100 simulations with 20 possibilities of interactions that will be evaluated through data provided at the end of each simulation (Gaillard, 2018; Morris et al., 2009).

At the end of the simulation, 20 positions of possible complexes are yielded, as criteria are evaluated the results of RMSD - Root Mean Square Deviation with values on the 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 (Shityakov & Förster, 2014). Thus, these parameters suggest values less than 2.0 for RMSD and result equal to or less than -6.0 kcal mol^{-1} for the binding energy (Morris et al., 2009).

Visualization of binding modes and protein-ligands interactions

In each simulation some software is used for visualization and image formatting, 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 (Pettersen et al., 2004; Salentin, Schreiber, Haupt, Adasme, & Schroeder, 2015).

Results and discussion

From the molecular docking simulations, analyzing the region of interest of each ligand, it can be seen in Figure 3 that all ligands have positional similarity in the formation of protein-ligand complexes.

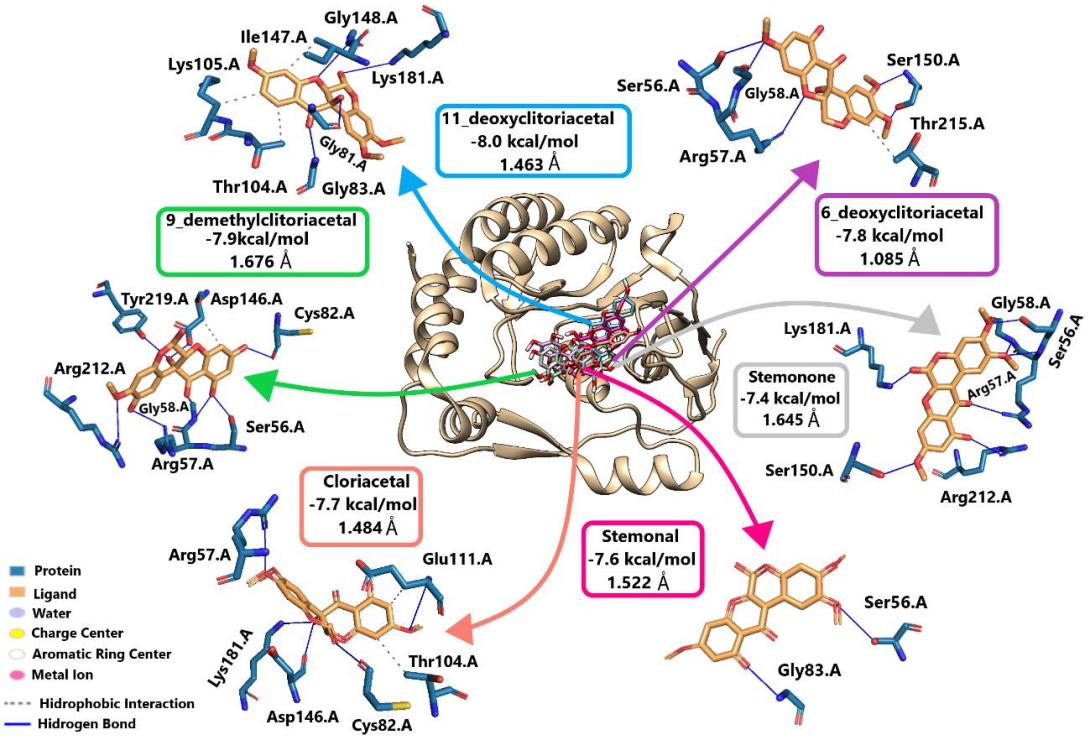

Figure 3. Three-dimensional formation of complexes between the protein (NS5MTaseDV) and ligands extracted from *Clitoria fairchildiana.*

Intrinsically, the compound 6_deoxyclitoriacetal found in Figure 3, exerted interactions with seven amino acids present in the NS5MTaseDV protein with a binding energy value of -7.8 kcal mol^{-1} and an RMSD of 1.085 Å, in its interactions the amino acids were (Ser56.A, Gly58.A, Arg57.A, Ser150.A, and Thr215.A), of all interactions only the amino acid Ser56.A is inserted in the active site region of the protein.

The ligand 9_demethylclitoriacetal in complex with the protein observed in figure X resulted in an energy of -7.9 kcal mol^{-1} and an RMSD equivalent to 1.676 Å, regarding its interactions the compound exhibits interactions with seven amino acids of the protein the residues are (Tyr219. A, Asp146.A, Cys82.A, Arg212.A, Gly58.A Ser56.A, and Arg57.A) of all the interacted amino acids only the residue Ser56.A integrates the site of interest of the protein.

The complex formed between 11_deoxyclitoriacetal with the protein has binding energy equivalent to -8.0 kcal mol^{-1} and an RMSD of 1.463 Å, as well as, interacting with only seven amino acids of the protein being the (Lys105. A, Ile147.A, Gly148.A, Lys181.A, Gly81.A, Gly83.A, and Thr104.A), of all interactions only Lys105.A, is part of the reference site of the studied protein.

The three-dimensional position of the Cloriacetal ligand and its respective interactions point out that the compound complexed close to the other ligands, having a value of (ΔG) of -7.7 kcal mol^{-1} and RMSD of 1. 484 Å and incorporated six interactions with amino acids (Arg57.A, Lys181.A, Asp146.A, Cys82.A, Thr104.A, and Glu111.A), referring to the site of interest the compound showed only one interaction that was performed with the residue Thr104.A.

Stemonal adhered in the region of interest similar to the others, its complex has an RMSD value of1.522Å and -7.6 kcal mol^{-1} binding energy; however, the ligand exerted only two interactions with the amino acids (Gly83.A and Ser56.A), of the interactions only the amino acid Ser56.A is part of the protein's site of interest.

Stemonone ligand showed the same structural behavior as the others when complexed with the protein with an energy value equivalent to -7.4 kcal mol^{-1} and 1. 645 Å related to RMSD; concerning its interactions it was demonstrated in figure x that the ligand interacted with six amino acids present in the target protein, the amino acids are: (Lys181.A, Gly58.A, Ser56.A, Arg57.A, Arg212.A, and Ser150.A), of all interactions only the residue Ser56.A, is part of the site of enzyme interactions.

All the interactions that the studied ligands exerted with the amino acids of the target protein, specifying all the distances along with the type of each bond, as exemplified in table 1.

Table 1. Distances of ligand-protein interactions

Compounds	Energy (kcal mol^{-1})	RMSD (Å)	Interactions	Bond type	Distance (Å)
6_deoxyclitoriacetal	-7.8	1.085	Thr215.A	Hydrophobic	3.94
			Ser56.A	H-Bond	2.42
			Arg57.A	H-Bond	3.17
			Gly58.A	H-Bond	3.68
			Ser150.A	H-Bond	2.66
			Ser150.A	H-Bond	2.20
9_demethylclitoriacetal	-7.9	1.676	Asp146.A	Hydrophobic	3.83
			Ser56.A	H-Bond	2.21
			Ser56.A	H-Bond	2.01
			Arg57.A	H-Bond	2.62
			Gly58.A	H-Bond	2.80
			Cys82.A	H-Bond	2.10
			Asp146.A	H-Bond	2.34
			Arg212.A	H-Bond	2.52
			Tyr219.A	H-Bond	3.32
11_deoxyclitoriacetal	-8.0	1.463	Thr104.A	Hydrophobic	3.77
			Lys105.A	Hydrophobic	3.36
			Ile147.A	Hydrophobic	3.61
			Gly81.A	H-Bond	2.11
			Gly83.A	H-Bond	2.93
			Gly148.A	H-Bond	2.88
			Lys181.A	H-Bond	3.10
Cloriacetal	-7.7	1.484	Thr104.A	Hydrophobic	3.91
			Glu111.A	Hydrophobic	3.47
			Arg57.A	H-Bond	2.56
			Cys82.A	H-Bond	2.63
			Glu111.A	H-Bond	3.60
			Asp146.A	H-Bond	2.12
			Lys181.A	H-Bond	3.13
Stemonal	-7.6	1.522	Ser56.A	H-Bond	3.37
			Gly83.A	H-Bond	2.77
Stemonone	-7.4	1.645	Ser56.A	H-Bond	1.86
			Arg57.A	H-Bond	2.26
			Arg57.A	H-Bond	2.78
			Gly58.A	H-Bond	2.43
			Ser150.A	H-Bond	2.84
			Lys181.A	H-Bond	2.39
			Arg212.A	H-Bond	2.85

The 6-deoxyclitoriacetal enabled six interactions with five amino acids, of these only one hydrophobic bond with Thr215.A at a distance equal to 3.94Å, in relation to hydrogen bonds five interactions were performed with the residues (Ser56. A, Arg57.A, Gly58.A, Ser150.A and Ser150.A), the smallest distance was in charge of the amino acid Ser150.A equal to 2.20Å, being only Ser56.A the amino acid present in the site of desired interactions with a distance equal to 2.42 Å.

9_demethylclitoriacetal exhibits eight hydrogen interactions and only one hydrophobic bond. Among the hydrogen interactions (Ser56.A, Ser56.A, Arg57.A, Gly58.A, Cys82.A, Asp146.A, Arg212.A, and Tyr219.A), the longest distance being 3.32 Å with the residue Tyr219.A and the shortest distance equivalent to 2.01 Å with the amino acid Ser56.A; the hydrophobic bond was performed by Asp146.A with a distance equal to 3.83Å. Of all the interactions performed only the amino acid Ser56.A is part of the active site of the protein.

Still analyzing Table 1, the interactions that the amino acids of the protein proposed with the formation of the complex through the ligand 11_deoxyclitoriacetal are exposed, thus, hydrogen and hydrophobic interactions were identified three hydrophobic interactions were exerted with the amino acids (Thr104.A, Lys105.A, and Ile147. A), with the best distance value of residue Lys105.A with its distance equal to 3.36Å; but hydrogen bonds were performed with the amino acids (Gly81.A, Gly83.A, Gly148.A, and Lys181.A), with the best distance equal to 2.11Å. Among the amino acids, residues Lys105.A and Thr104.A is part of the active site of the protein.

The Cloriacetal presents in table 1, establishes seven interactions with six amino acids two hydrophobic interactions with residues (Thr104.A and Glu111.A) and possessing their respective distances of 3.91Å and 3. 47Å; as for hydrogen bonds, the interactions with (Arg57.A, Cys82.A, Glu111.A, Asp146.A and Lys181.A) are pointed out. Of all the interactions, the one that stands out is Thr104.A, for being an amino acid present in the active site.

In complex with the protein the compound Stemonal presents two interactions with amino acids (Ser56.A and Gly83.A), both hydrogen bonds with respective distance values of 3.37Å and 2.77Å. Regarding the distance, it is pointed out the amino acid Gly83.A, because it had the shortest distance, but Ser56.A belongs to the reference amino acids of the target protein.

The ligand Stemonone, observed in table 1, has seven hydrogen bonds with the amino acids (Ser56.A, Arg57.A, Arg57.A, Gly58.A, Ser150.A, Lys181.A, and Arg212.A) of all the interactions the longest distance was with the residue Arg212. A with a distance of 2.85Å, but the shortest distance was realized with the amino acid Ser56.A with its distance equivalent to 1.86 Å, this amino acid is present in the active site of the protein.

Considering the literature, the rotenoids extracted from *C. fairchildiana* corroborate the in silico data, (Bertonceli et al., 2022) described in their research that the rotenoids (6-deoxyclitoriacetal and 11-deoxyclitoriacetal) have a high toxicity rate in Aedes aegypti mosquito larvae, leading to changes in the exoskeleton, cuticular detachment and perforations in the larval thorax and abdomen, interference in the acidification process of the cellular vesicles in the larvae's midgut and, due to the significant increase in the production of reactive oxygen species (ROS) in the larvae, they trigger a process of oxidative stress in these insects.

Likewise, A Shaalan and V Canyon (2015) establish that *C. fairchildiana* rotenoids extracted from the essential oils of the seeds demonstrate a range of larvicidal, adulticidal, growth-regulating, ovicidal, oviposition deterrent and repellent activities. The lethal concentrations and toxicity of more highly active botanicals are comparable to those of organophosphates, making them potential candidates for future laboratory investigations and field evaluations.

Conclusion

From the complexes formed between protein-ligands based on in silico model with the help of molecular docking simulation between the ligands (6_deoxyclitoriacetal, 9_demethylclitoriacetal, 11_deoxyclitoriacetal, Cloriacetal, Stemonal, and Stemonone), with dengue protein (NS5MTaseDV), it was observed that all the ligands possessed great values regarding binding energy, where the highest energy was realized by the ligand Stemonone with its ΔG equal to -7. 4 kcal mol^{-1}, and the optimal energy with the lowest energy value equal to -8. 0 kcal mol^{-1} performed from the compound 11_deoxyclitoriacetal, about the RMSD the best value was obtained through the ligand 6_deoxyclitoriacetal with 1.085Å, of all the interactions performed by the ligands (6_deoxyclitoriacetal, Cloriacetal, Stemonal, and Stemonone) interacted with only one amino acid present in the reference site of the protein, the compound (9_demethylclitoriacetal) has two interactions with the amino

acid Ser56. A, a residue present in the active site of the protein but the ligand (11_deoxyclitoriacetal) has two interactions with the amino acids Thr104 and Lys105, both amino acids present in the interaction site of the protein. Thus, it is inferred that the compounds evaluated in this study may have inhibitory potential against the dengue protein (NS5MTaseDV), especially the compounds (9_demethylclitoriacetal and 11_deoxyclitoriacetal), by presenting in the analyses two interactions with the protein reference residues.

Because of this, there is a lack of *in vivo* and *in silico* studies on the potential of the rotenoids extracted from the *C. fairchildiana* plant, which could be a broad aspect of scientific research into testing them against neglected diseases, providing new insights into public health.

Acknowledgments

Universidade Estadual do Ceará - UECE for promoting teaching, research and extension aiming at the interdisciplinary learning of its students, *Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico* (FUNCAP) for granting the scholarship, *Grupo de Química e Eletroquímica* (GQTE) for the cooperation, planning and execution of the scientific projects and to the other authors for the trust and activities carried out for the completion of the manuscript.

References

A Shaalan, E., & V Canyon, D. (2015). A review on mosquitocidal activity of botanical seed derivatives. *Current Bioactive Compounds*, *11*(2), 78-90. Retrieved from https://www.ingentaconnect.com/content/ben/cbc/2015/00000011/00000002/art00005

Alves, M. M., Alves, E. U., & Santos-Moura, S. D. S. (2015). Physiological quality seeds of *Clitoria fairchildiana* RA Howard during storage. *Bioscience Journal*, *31*(3), 767-774. DOI: https://doi.org/10.14393/BJ-v31n3a2015-26085

Annegowda, H. V., Bhat, R., Tze, L. M., Karim, A. A., & Mansor, S. M. (2013). The free radical scavenging and antioxidant activities of pod and seed extract of *Clitoria fairchildiana* (Howard)-an underutilized legume. *Journal of Food Science and Technology*, 50, 535-541. DOI: https://doi.org/10.1007/s13197-011-0370-8

Araújo, V. E. M. D., Bezerra, J. M. T., Amâncio, F. F., Passos, V. M. D. A., & Carneiro, M. (2017). Aumento da carga de dengue no Brasil e unidades federadas, 2000 e 2015: Análise do global burden of disease study 2015. *Revista Brasileira de Epidemiologia*, *20*(1), 205-216. DOI: https://doi.org/10.1590/1980-5497201700050017

Barroso, I. L. D., Santos Soares, A. G., Silva Soares, G., Viana, J. A., Lima, L. N. F., Conceição Sousa, M., ... & Moura Diniz, R. (2020). A study on the prevalence of dengue fever in Brazil: Analysis of the literature. *Brazilian Journal of Development*, *6*(8), 61878-61883. DOI: https://doi.org/10.34117/bjdv6n8-565

Bertonceli, M. A. A., Oliveira, A. E. A., Souza Passos, M., Vieira, I. J. C., Braz-Filho, R., Lemos, F. J. A., ... & Fernandes, K. V. S. (2022). Rotenoids from *Clitoria fairchildiana* R. Howard (Fabaceae) seeds affect the cellular metabolism of larvae of *Aedes aegypti* L. (Culicidae). *Pesticide Biochemistry and Physiology*, 186. DOI: https://doi.org/10.1016/j.pestbp.2022.105167

Carneiro, A. C. A., Reis, A. L. A., Silveira, P. F., Ribeira, E. M., Ferreira, C. S., & BM, S. (2015). Análise do envolvimento da proteína ns1 de dengue vírus na modulação da atividade transcricional no promotor de il-6 em células hepáticas humanas. *Blucher Biochemistry Proceedings*, *1*(1), 68-69. DOI: https://doi.org/10.5151/biochem-jaibqi-0007

Codding, P. W. (2013). *Structure-based drug design: experimental and computational approaches (Vol. 352)*. Springer Science & Business Media. DOI: https://doi.org/10.1007/978-94-015-9028-0

Costa, L. G., Silva, A. G., & Gomes, D. R. (2014). Morphology of fruits, seeds and seedlings and anatomy of seeds of sombreiro (*Clitoria fairchildiana*). *Revista de Ciências Agrárias/Amazonian Journal of Agricultural and Environmental Sciences*, *57*(4), 414-421. DOI: https://doi.org/10.4322/rca.1596

Dewick, P. M. (2017). Isoflavonoids. In *The Flavonoids Advances in Research Since 1986* (p. 117-238). New York, US: Routledge. DOI: https://doi.org/10.1201/9780203736692

Egloff, M. P., Benarroch, D., Selisko, B., Romette, J. L., & Canard, B. (2002). An RNA cap (nucleoside-2′-O-)-methyltransferase in the *flavivirus* RNA polymerase NS5: Crystal structure and functional characterization. *The EMBO Journal*, *21*(11), 2757-2768. DOI: https://doi.org/10.1093/emboj/21.11.2757

Fokoue, H. H., Pinheiro, P. S., Fraga, C. A., & Sant'Anna, C. M. (2020). Há algo novo no reconhecimento molecular aplicado à química medicinal? *Química Nova*, *43*(1), 78-89. DOI: https://doi.org/10.21577/0100-4042.20170474

Furuya-Kanamori, L., Liang, S., Milinovich, G., Soares Magalhaes, R. J., Clements, A. C., Hu, W., ... Yakob, L. (2016). Co-distribution and co-infection of chikungunya and dengue viruses. *BMC infectious diseases*, *16*(84), 1-11. DOI: https://doi.org/10.1186/s12879-016-1417-2

Gaillard, T. (2018). Evaluation of AutoDock and AutoDock Vina on the CASF-2013 benchmark. *Journal of chemical Information and Modeling*, *58*(8), 1697-1706. DOI: https://doi.org/10.1021/acs.jcim.8b00312

García, G., González, N., Pérez, A. B., Sierra, B., Aguirre, E., Rizo, D., ... Guzmán, M. G. (2011). Long-term persistence of clinical symptoms in dengue-infected persons and its association with immunological disorders. *International Journal of Infectious Diseases*, *15*(1), e38-e43. DOI: https://doi.org/10.1016/j.ijid.2010.09.008

Gubler, D. J. (2019). Dengue. In *The arboviruses: Epidemiology and ecology* (p. 223-260). Boca Raton, FL: CRC Press. DOI: https://doi.org/10.1201/9780429280245

Halgren, T. A. (1996). Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *Journal of Computational Chemistry*, *17*(5), 490-519. DOI: https://doi.org/10.1002/(SICI)1096-987X(199604)17:5/6<490::AID-JCC1>3.0.CO;2-P

Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. (2012). Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics*, *4*(1), 1-17. DOI: https://doi.org/10.1186/1758-2946-4-17

Harapan, H., Michie, A., Sasmono, R. T., & Imrie, A. (2020). Dengue: A minireview. *Viruses*, *12*(8), 829. DOI: https://doi.org/10.3390/v12080829

Harapan, H., Ryan, M., Yohan, B., Abidin, R. S., Nainu, F., Rakib, A., ... Sasmono, R. T. (2021). Covid-19 and dengue: Double punches for dengue-endemic countries in Asia. *Reviews in Medical Virology*, *31*(2). DOI: https://doi.org/10.1002/rmv.2161

Hotez, P. J., Alvarado, M., Basáñez, M. G., Bolliger, I., Bourne, R., Boussinesq, M., ... Naghavi, M. (2014). The global burden of disease study 2010: Interpretation and implications for the neglected tropical diseases. *PLoS neglected tropical diseases*, *8*(7). DOI: https://doi.org/10.1371/journal.pntd.0002865

Jakhar, R., Dangi, M., Khichi, A., & Chhillar, A. K. (2020). Relevance of molecular docking studies in drug designing. *Current Bioinformatics*, *15*(4), 270-278. DOI: https://doi.org/10.2174/1574893615666191219094216

Konozy, E., Osman, M., & Dirar, A. (2022). Plant lectins as potent Anti-coronaviruses, Anti-inflammatory, antinociceptive and antiulcer agents. *Saudi Journal of Biological Sciences*, *29*(6). DOI: https://doi.org/10.1016/j.sjbs.2022.103301

Lima, A. N., Philot, E. A., Trossini, G. H. G., Scott, L. P. B., Maltarollo, V. G., & Honorio, K. M. (2016). Use of machine learning approaches for novel drug discovery. *Expert Opinion on Drug Discovery*, *11*(3), 225-239. DOI: https://doi.org/10.1517/17460441.2016.1146250

Lowe, R., Barcellos, C., Brasil, P., Cruz, O. G., Honório, N. A., Kuper, H., & Carvalho, M. S. (2018). The Zika virus epidemic in Brazil: from discovery to future implications. *International Journal of Environmental Research and Public Health*, *15*(1), 96. DOI: https://doi.org/10.3390/ijerph15010096

Maia, E. H. B., Assis, L. C., Oliveira, T. A., Silva, A. M., & Taranto, A. G. (2020a). Structure-based virtual screening: From classical to artificial intelligence. *Frontiers in Chemistry*, *8*, 343. DOI: https://doi.org/10.3389/fchem.2020.00343

Maia, E. H., Medaglia, L. R., Silva, A. M., & Taranto, A. G. (2020b). Molecular architect: A user-friendly workflow for virtual screening. *ACS omega*, *5*(12), 6628-6640. DOI: https://doi.org/10.1021/acsomega.9b04403

Mathias, L., Da Silva, B. P., Mors, W. B., & Parente, J. P. (2005). Isolation and structural elucidation of a novel rotenoid from the seeds of *Clitoria fairchildiana*. *Natural Product Research*, *19*(4), 325-329. DOI: https://doi.org/10.1080/14786410412331272022

Mathias, L., Mors, W. B., & Parente, J. (1998). Rotenoids from seeds of *Clitoria fairchildiana*. *Phytochemistry, 48*(8), 1449-1451. DOI: https://doi.org/10.1016/S0031-9422(97)00933-3

Medeiros Filho, F. C., dos Santos Nascimento, K., Santos, W. O., & Frazão, N. F. (2020). Estudo da inibição da acetilcolinesterase por docking molecular: Aplicação no tratamento da doença do Alzheimer. *Educação, Ciência e Saúde*, *7*(2), 18. DOI: https://doi.org/10.20438/ecs.v7i2.297

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785-2791. DOI: https://doi.org/10.1002/jcc.21256

Oliveira, R. D. M. A. B., Araújo, F. M. D. C., & Cavalcanti, L. P. D. G. (2018). Aspectos entomológicos e epidemiológicos das epidemias de dengue em Fortaleza, Ceará, 2001-2012. *Epidemiologia e Serviços de Saúde*, *27*(1), e201704414. DOI: https://doi.org/10.5123/S1679-49742018000100014

Pereira da Silva, B., & Paz Parente, J. (2002). Antiinflammatory activity of rotenoids from *Clitoria fairchildiana*. *Phytotherapy Research*, *16*(1), 87-88. DOI: https://doi.org/10.1002/ptr.807

Pescarini, J. M., Rodrigues, M., Paixão, E. S., Cardim, L., Brito, C. A. D., Costa, M. D. C. N., ... Brickley, E. B. (2022). Dengue, Zika, and Chikungunya viral circulation and hospitalization rates in Brazil from 2014 to 2019: An ecological study. *PLoS Neglected Tropical Diseases*, *16*(7), e0010602. DOI: https://doi.org/10.1371/journal.pntd.0010602

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera - A visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, *25*(13), 1605-1612. DOI: https://doi.org/10.1002/jcc.20084

Piccirillo, E., & Amaral, A. T. D. (2018). Busca virtual de compostos bioativos: Conceitos e aplicações. *Química Nova*, *41*(6), 662-677. DOI: https://doi.org/10.21577/0100-4042.20170210

Pitakpawasutthi, Y., Suwatronnakorn, M., Issaravanich, S., Palanuvej, C., & Ruangrungsi, N. (2019). Quality evaluation with reference to clitoriacetal and in vitro antioxidant activities of *Clitoria macrophylla* root. *Journal of Advanced Pharmaceutical Technology & Research*, *10*(4), 169. DOI: https://doi.org/10.4103/japtr.japtr_67_19

Salentin, S., Schreiber, S., Haupt, V. J., Adasme, M. F., & Schroeder, M. (2015). PLIP: Fully automated protein–ligand interaction profiler. *Nucleic Acids Research*, *43*(W1), W443-W447. DOI: https://doi.org/10.1093/nar/gkv315

Santos, R. A., David, J. M., & David, J. P. (2016). Detection and quantification of rotenoids from *Clitoria fairchildiana* and its lipids profile. *Natural Product Communications*, *11*(5), 631-632. DOI: https://doi.org/10.1177/1934578X1601100519

Santos, R. D. C., Daniel, I. C., Próspero, D. F. A., & Costa, C. L. S. (2018). Modificação molecular incremental: Análise de parâmetros físico-químicos, farmacocinéticos e toxicológicos in sílico de fármacos inibidores seletivos da recaptação de serotonina (ISRSs). *Boletim Informativo Geum*, *9*(2), 31. Retrieved from https://revistas.ufpi.br/index.php/geum/article/view/6347

Shityakov, S., & Förster, C. (2014). *In silico* predictive model to determine vector-mediated transport properties for the blood–brain barrier choline transporter. *Advances and Applications in Bioinformatics and Chemistry: AABC*, *7*, 23-36. DOI: https://doi.org/10.2147/AABC.S63749

Terra, M. R., Da Silva, R. S., Pereira, M. G. N., & Lima, A. F. (2017). *Aedes aegypti* e as arbovíroses emergentes no Brasil. *Revista Uningá Review*, *30*(3), 52-60. Retrieved from https://revista.uninga.br/uningareviews/article/view/2028

Wilder-Smith, A., Ooi, E. E., Horstick, O., & Wills, B. (2019). Dengue. *The Lancet*, *393*(10169), 350-363. DOI: https://doi.org/10.1016/S0140-6736(18)32560-1

Zara, A. L. D. S. A., Santos, S. M. D., Fernandes-Oliveira, E. S., Carvalho, R. G., & Coelho, G. E. (2016). Estratégias de controle do *Aedes aegypti*: Uma revisão. *Epidemiologia e Serviços de Saúde*, *25*, 391-404. DOI: https://doi.org/10.5123/S1679-49742016000200017