Molecular coupling study between the potential inhibitor of dengue fever, Annatto and Protein E (DENV-4)

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Abstract— Dengue fever is a viral disease belonging to the family Flaviviridae, genus Flavivirus, and this genus consists of more than 70 viruses. Most are transmitted by arthropod vectors, so these viruses are also called arboviruses. The transmission of dengue occurs with higher incidence in tropical countries, whose environmental conditions are characterized by high rainfall rates and elevated temperatures, guaranteeing the reproduction and development of the mosquito Aedes aegypti. Therefore, because these characteristics are present, Brazil is one of the countries that favors the existence of the vector and, consequently, the emergence of arboviruses. In this perspective, the development of methods to reduce mosquito propagation is the main means of control, in which the vector is the primordial element in the transmission chain of the virus. Among the several techniques being studied for the control of Aedes aegypti is the development of new formulas based on active plant principles, in which the use of photosensitizing substances (FSs) or photo insecticides is a favorable tool to combat Insect-vectors. Bixin is a carotenoid found in the *Bixaorellana* L. plant, better known in Brazil as annatto, belonging to the family Bixaceae, genus Bixa. This plant is evidenced by producing a reddish pigment, taken from its seeds, which was constantly used by the Indians as a protector (repellent) for skin against insect bites. Therefore, this insect repellency was proven by means of scientific tests. Therefore, from Bixin, pigments are obtained: Norbixin, Annatto and seven more pigments, both of which are Bixin isomers. Thus, the objective of this work was to carry out characterization and molecular docking studies using the Annatto ligand and the 5B1C protein (PDB code) of the dengue virusserotype 4 (DENV-4). In this perspective, the Annatto structure was analyzed using ChemSketch® software, where it was possible to characterize the molecule with respect to molecular formula, molar mass, percentage composition, molar refraction, molar volume, refractive index, surface tension, density, polarizability, Nominal mass and calculated mass. Subsequently, the ligand was submitted to the semi-empirical calculations of the Arguslab® software, where its structure was converged and optimized. Then, the docking between Annatto and the DENV - 4 protein was performed using the UCSF Chimera ® software. After termination of the molecular coupling, the deviation between the ligand and the 5B1C protein was analyzed, where RMSD values (Root mean square deviation) between the structures were calculated. The following values are found, RMSD lb: 1.994 Å and RMSD ub: 2.409 Å, Score of -6.4 and 12 active twists, the ligand was connected to the amino acids Arginine 143 and Lysine 235, located in chain A and C, already in Score -6.5, Annatto was ligated to the amino acid Arginine 95, being this located in the chain B. Nevertheless, both scores produced 12 active twists in the software UCSF Chimera®. It was also possible to observe that the O2 atom of the linker is closer to the amino acid Arginine143, located in the A chain of the DENV-4 protein at 2.2 Å of distance, indicate that the ligand-protein complex is stable, and that It offers great possibilities of being used in the development of new active compounds against this disease.

Keywords: Annatto. Dengue Fever. DENV-4. Molecular Docking.Protein E.

1 INTRODUCTION

Dengue is a viral disease belonging to the family Flaviviridae, genus Flavivirus, being this genus constituted by more than 70 viruses [1]; In which the Flaviviruses are enveloped viruses of icosahedral symmetry and measure from 40 to 60 nm [2], most of which are transmitted by arthropod vectors, therefore, these viruses are also called arboviruses [1].However, the transmission of dengue occurs with higher incidence in tropical countries, whose environmental conditions are characterized by high rainfall rates and elevated temperatures, guaranteeing the reproduction and development of the Aedes aegypti mosquito [3]. Therefore, because of these characteristics, Brazil is one of the countries that favors the existence of the vector and, consequently, the emergence of arboviruses [4]. In view of this, 2016 was marked by large outbreaks of dengue worldwide, where the Region of the Americas registered more than 2.38 million cases, in which only Brazil contributed 1.5 million cases. This number approximately 3 times higher than in 2014 [5].

In this perspective, human beings are the main hosts of the dengue virus, in which its transmission occurs through the bite of females of the mosquito Aedes aegypti [6], being this mosquito coming from the African continent; Arriving to Brazil in the colonial period in slave ships [7]. Where the virus incubation period is 4 to 10 days, in which an infected mosquito can propagate the virus throughout its life [8], and Aedes aegypti also transmits chikungunya, yellow fever and Zika [5].The

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dengue virus (DENV) presents distinct antigenic characteristics characterizing dengue as the only human arboviruses that can be caused by four different serotypes, DENV 1, DENV 2, DENV 3 and DENV 4 [9], [10] being the latter identified in 1981 in the Caribbean Islands and spread by several American countries [11]. Considering that the first report of reintroduction of DENV-4 in Brazil occurred in the state of Manaus, in 2008 [12]. However, epidemics were recorded only as of 2010 in Rondônia, a state located in the northern region of the country [13].

By means of crystallographic studies it was possible to identify that the Dengue Virus E protein (envelope) has three domains: the first (I) central, the second (II) containing the dimerization region and the fusion peptide, and the third III), which exhibits the receptor binding site [14]. Among them, the domain III (ED3) is evidenced, since it has residues responsible for the determination of tropism and virulence among the Flavivirus [15]. Therefore, ED3 protects against the four serotypes of DENV including against other Flaviviruses [16] [17] [18].Dengue virus E protein is glycosylated and forms homodimers, each monomer consisting of their respective domains [19] related to virus binding on the host cell membrane, in addition to providing protective immune responses [20], [15]. Thus, protein E, is the major and major structural protein of Flavivirus, being responsible for the biological activities of the viral cycle, such as the assembly of the viral particle, interaction with cellular receptors and membrane binding, besides being the Main target of neutralizing antibodies and have hemagglutinating activity [2], [21]. In this perspective, protein E has the highest target of humoral immunity, in that it induces the production of antibodies [22].

Therefore, the entry of dengue viruses into the cell is performed by the interaction of the envelope protein with cellular receptors present on the surface of target cells, in addition to heparan sulfate molecules that act as co-receptors in this process 23. Therefore, the development of methods to decrease mosquito propagation is the main means of control, in which the vector is the primordial element in the virus transmission chain [24]. Among the several techniques being studied for the control of Aedes aegypti is the development of new formulas based on active plant principles, in which the use of photosensitizing substances (FSs) or photo insecticides is a favorable tool to combat Insect-vectors [25] [26] [27].

It is noteworthy that the role of these substances (FSs) have been researched as potential progenies of traditional insecticides to control the emergence of pests, as well as combating mosquito vectors [28]. Considering that secondary plant metabolites with repellency, growth regulating and insecticidal properties are one of the alternatives for the control of A. aegypti [29]. Thus, plant extracts are sources of bioactive substances that may influence insect behavior such as repellency, inhibition of egg deposition and feeding, larval growth disorders, deformations, infertility and mortality [29].

Bixin is a carotenoid found in the *Bixaorellana* L. plant, better known in Brazil as annatto, belonging to the family Bixaceae, genus Bixa. This plant is evidenced by producing a reddish pigment, removed from its seeds, which was constantly used by the Indians as a protector (repellent) for skin against insect bites [30]. Thus, this insect repellency has been proven by scientific testing [31]. Therefore, from bixin, pigments are obtained: Norbixin, Annatto and seven more pigments, both of which are Bixin isomers. Despite the low investment in research on medicinal plants, it is estimated that at least half of the plants contain substances called active ingredients, which have curative and preventive properties for many diseases [32]. In this perspective, the objective of this work was to carry out characterization and molecular docking studies using the Annatto ligand and the 5B1C protein (PDB code) of the dengue virus, more specifically DENV-4.

2METHODOLOGY

2.1. Computer Resources

For this work, ACD / Labs ChemSketch®, Arguslab® and USF-Chimera® software were used, which are free of charge, based on the Windows 7 Ultimate 64 Bit Operating System with Intel® Core ™ i3-5005U CPU @ 2.0 GHz, 4 GB of RAM .

2.2. Obtaining the molecular structure of the Annatto ligand and the DENV-4 protein

From the ChemIdplus repository (https://chem.nlm.nih.gov/chemidplus/) the ligand structure with registration number 1393-63-1 was obtained. Then the annatto molecule was characterized using ChemSketch® Freeware license software belonging to the ACD / Labs package. The crystalline structure of the DENV-4 ED3 mutant protein with L387I PDB code (5B1C) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) repository, A database for 131485 biological macromolecular structures [33].

2. 3.Preparation of Ligand

With the obtaining of the ligand structure it was possible to perform the optimization and characterization of the same, using Arguslab® software [34]; (QM-AM1) using the Hatree-Fock SCF (200 max.interations, convergence 10^{-10} kcal mol^{-1}, RHF-closed shell) algorithm.

2. 4.Preparation of DENV-4 Protein

Protein preparation for molecular docking was performed by the UCSF Chimera® software. [35] Freeware license, in which were removed the residues (H2O and SO4) that could influence the satisfactory result of the coupling [36].

2. 5.Docking Molecular

After preparation of the DENV-4 ligand and protein, molecular docking was performed using the UCSF Chimera® software. Using the flexible ligand and the rigid protein [36].

3 RESULTS AND DISCUSSIONS

ChemSketch® software belongs to the ACD / Labs package, which allows you to design various chemical structures as well as calculate their respective basic molecular properties. In this perspective, the Annatto structure was analyzed using ChemSketch® software, where it was possible to characterize the molecule with respect to molecular formula, molar mass, percentage composition, molar refraction, molar volume, refractive index, surface tension, density, polarizability,nominal mass and calculated mass (Figure 1).

Molecular Formula	= $C_{24}H_{28}O_4$
Formula Weight	= 380.47672
Composition	= C(75.76%) H(7.42%) O(16.82%)
Molar Refractivity	= 116.25 ± 0.3 cm^3
Molar Volume	= 355.6 ± 3.0 cm^3
Parachor	= 900.2 ± 4.0 cm^3
Index of Refraction	= 1.567 ± 0.02
Surface Tension	= 41.0 ± 3.0 dyne/cm
Density	= 1.069 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 46.08 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 380.198759 Da
Nominal Mass	= 380 Da
Average Mass	= 380.4767 Da

Fig.1. Molecular properties of Annatto obtained from ChemSketch software.

In Figure 2, it is possible to observe the two-dimensional structure of the ligand, which is found in the ChemIdplus repository.

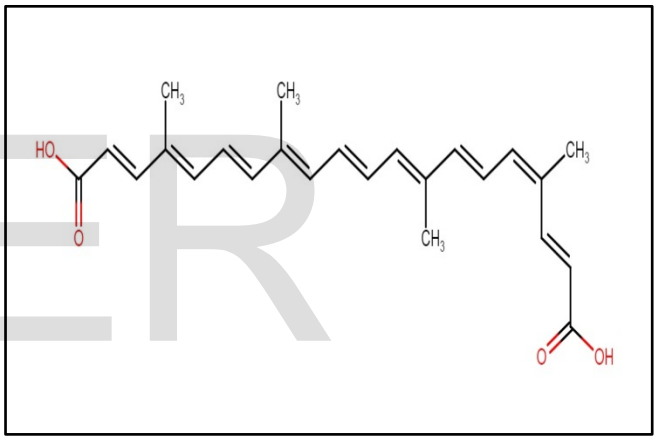

Fig.2. Annatto molecular structure obtained from the ChemIdplus repository (registration number 1393-63-1).

Using the ChemSketch® software, it was possible to visualize the 3D structure of the ligand (Figure 3) in which carbon atoms are represented by turquoise blue, hydrogens are arranged in white and red are oxygen atoms.

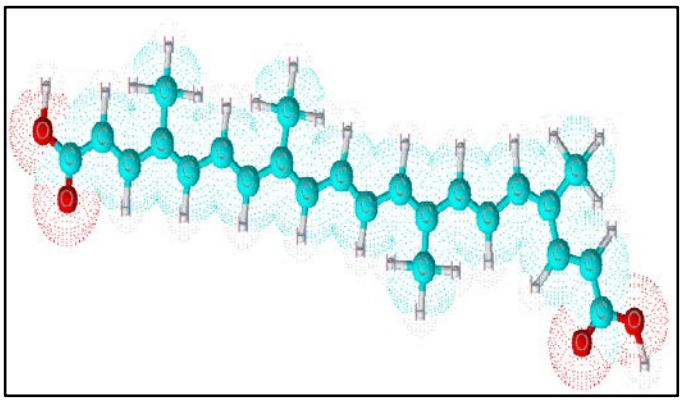

Fig.3. 3D structure of Annatto, Displayed by the ChemSketch® software.

Subsequently, the ligand was submitted to the semi-empirical calculations of the Arguslab® software, where its structure was converged and optimized (Figure 4).

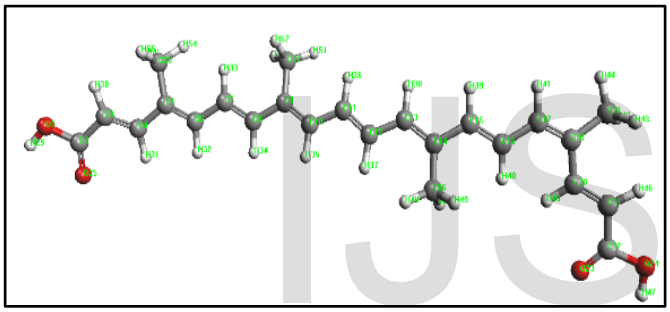

Fig.4. Annatto molecular structure optimized by Arguslab® software.

In figure 5 we can visualize the molecular docking between the Annatto ligand and the DENV-4 protein run in the UCSF Chimera® software. After termination of the molecular coupling, the deviation between the ligand and the 5B1C protein was analyzed, where the RMSD values (mean square root deviation) between the structures were calculated [36]. The following values are found; RMSD l.b: 1.994 Å and RMSD u.b: 2.409 Å, Score of -6.4 and 12 active twists. It was also possible to observe that the O2 atom of the linker is closest to the amino acid ARG 143 (Arginine), located in the A chain (represented by the green color) of the DENV-4 protein at 2.2Å.

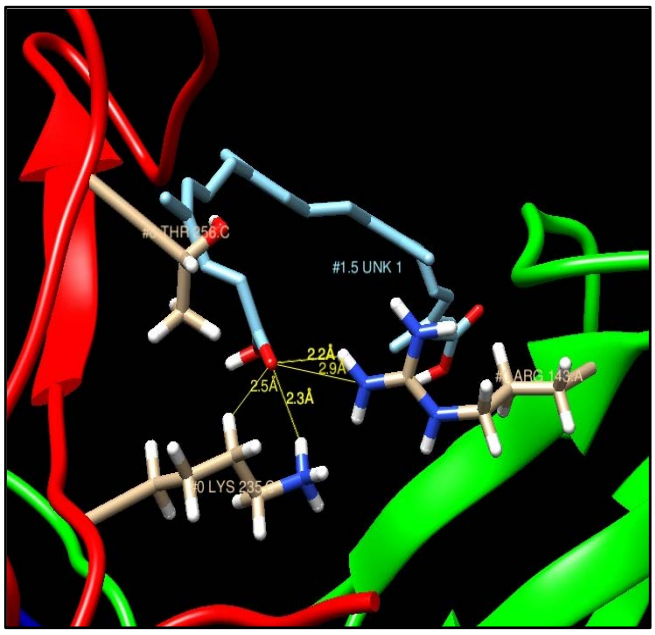

Fig.5. Molecular docking between Annatto ligand and DENV-4 envelope protein, using UCSF Chimera® software.

In figure 6 it is possible to observe the distances existing between the oxygen atom two (O2) and the amino acids of the chain A and C. In that the smaller distances found were of 2.2 Å between the ligand and the Arginine 143 of the chain A and 2.3 Å Distance between the linker and the amino acid Lysine 235 of the protein located in the C chain, in which the A chain is represented by the green ribbons and the C chain by the red ribbons, shown previously in Figure 5.

ID	Atom 1	Atom 2	Distance
1	#1.5 UNK 1 O2	#0 LYS 235.C CD	3.4Å
2	#1.5 UNK 1 O2	#0 LYS 235.C HZ2	2.3Å
3	#1.5 UNK 1 O2	#0 ARG 143.A NH2	2.9Å
4	#1.5 UNK 1 O2	#0 LYS 235.C NZ	3.3Å
5	#1.5 UNK 1 O2	#0 ARG 143.A HH21	3.0Å
6	#1.5 UNK 1 O2	#0 THR 256.C HG21	3.5Å
7	#1.5 UNK 1 O2	#0 LYS 235.C HD3	2.5Å
8	#1.5 UNK 1 O2	#0 ARG 143.A HH22	2.2Å

Fig.6. Distances found between the Annatto ligand and the DENV - 4 Protein, making use of the UCSF Chimera ® software.

The receptor site is characterized as to its ability to bind molecules by using specific functional groups selected by the software from its own database, where such clusters serve to search for fragments of the linker Can bind satisfactorily with the amino acids of the receptor site, generating a new binding molecule [37]. Thus, Figure 7 demonstrates the ligand-receptor site interaction between Annatto and DENV-4 protein, which is observed in the hydrophilic region (represented by the blue color).

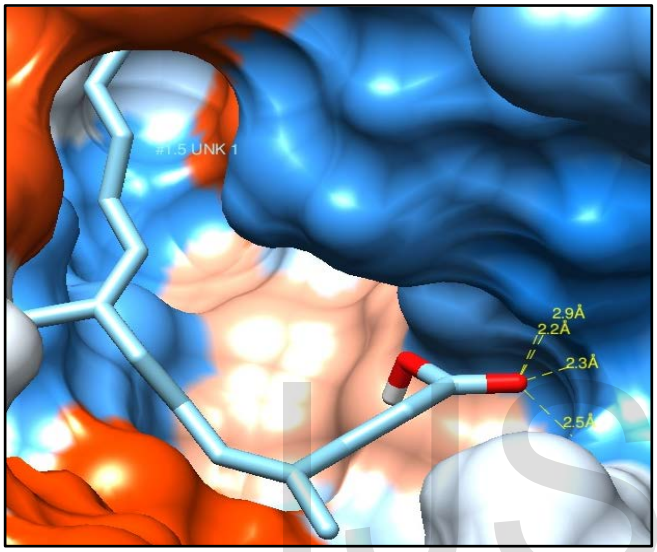

Fig.7. Interaction of the Annatto linker to the receptor site of the protein.

The molecular docking methods, for the most part, involve a power function containing electrostatic, van der Waals, hydrogen bonding and sometimes hydrophobic parameters, and these parameters produce mathematical models that predict the best orientations of the ligand, According to a list of energy scores [37]. In this perspective, Figure 8 shows the lowest H (Hydrogen) binding found between the receptor-ligand complex, which is in another docking position, with RMSD values lb: 21.787Å and RMSD ub: 24.986 Å, 6.5 and 12 active twists. Where the H19 atom of the linker is closest to the amino acid ARG (Arginine) 95 located in the B chain (represented by blue) at 2.0 Å distance.

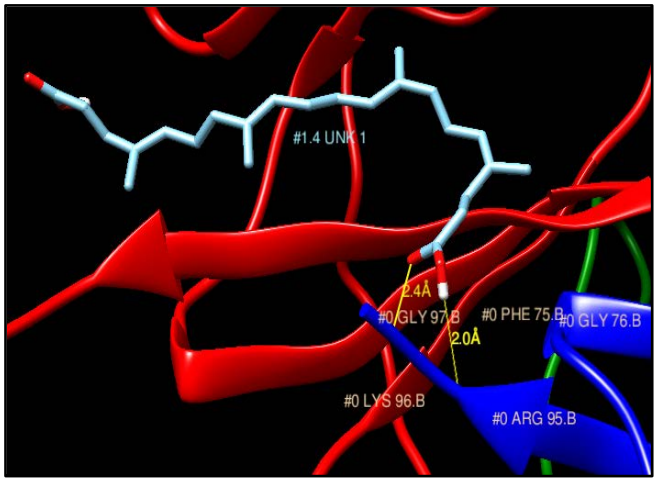

Fig.8. Lower Hydrogen binding found between Annatto ligand and DENV Protein using UCSF Chimera® software.

After analyzing the obtained results, it was possible to notice that in the two docking scores analyzed, the Annatto ligand was coupled in different chains and amino acids of the DENV-4 protein, that is, in Score -6.4 the ligand was connected to amino acids Arginine 143 and Lysine 235, located in the chain A and C, already in the Score -6.5, Annatto bound to the amino acid Arginine 95, being this located in the chain B. Nevertheless, both scores produced 12 active twists in the software UCSF Chimera®.

4 CONCLUSIONS

The present work focused on the characterization and molecular docking studies using the Annatto ligand and the 5B1C protein (PDB code) of the dengue virusserotypes 4, (DENV-4). In this perspective, the Annatto structure was analyzed using ChemSketch® software, where it was possible to characterize the molecule with respect to molecular formula, molar mass, percentage composition, molar refraction, molar volume, refractive index, surface tension, density, polarizability, Nominal mass and calculated mass. Subsequently, the ligand was submitted to the semi-empirical calculations of the Arguslab® software, where its structure was converged and optimized. Then, the docking between Annatto and the DENV - 4 protein was performed using the UCSF Chimera ® software. After termination of the molecular coupling, the deviation between the ligand

and the 5B1C protein was analyzed, where RMSD values (Root mean square deviation) between the structures were calculated. The following values are found, RMSD lb: 1.994 Å and RMSD ub: 2.409 Å, score of -6.4 and 12 active twists, the Annatto linker was coupled to different chains and amino acids of the DENV-4 protein, i.e., on Score -6.4, the linker was Connected to the amino acids Arginine 143 and Lysine 235, located in the chain A and C, already in Score -6.5, Annatto bound to the amino acid Arginine 95, being this located in the chain B. Nevertheless, both scores produced 12 active twists in the software UCSF Chimera®.

It was also possible to observe that the O2 atom of the linker is closer to the amino acid ARG 143 (Arginine), located in the A chain of the DENV-4 protein at 2.2 Å of distance, indicate that the ligand-protein complex is stable, and that It offers great possibilities of being used in the development of new compounds against this disease.

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