Study of the interactional properties between Curcumin / Monodimethylcurcumin andprotein (NS1) of dengue fever virus type 4 (DENV4)

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Abstract—Aedes aegypti, a mosquito that transmits dengue fever, belongs to the Flaviviridae family, the genus Flavivirus, a viral disease caused by arboviruses of the hematophagous species, currently has four serotypes DEN-1, DEN-2, DEN-3 and DEN-4. About 390 million people a year in the world are infected with the dengue virus. The mature virus has a length of approximately 11 kilobases (Kb), coated with an icosahedral protein coat (capsid), after cleavage it generates three structural proteins and seven non-structural proteins. The human being has always made use of medicinal plants for therapeutic purposes to seek better responses to treatments. The Ligand curcumin or turmeric, a native species of Indian shrub has been registered since centuries in both cooking and Indian medicine, which has antioxidant pharmacological actions attributed to its hydroxyl and methoxy groups, characterized by its anti-inflammatory action, Adept at antibacterial, antiviral, antifungal and antitumor action. The present study was developed using the molecular anchoring methodology(Docking), with the objective of evaluating the interaction between the Curcumin ligand and the dengue serotype four virus (DENV-4) protein(NS1), through computational simulation. The molecular docking results showed a correlation with the activity values of the compounds, confirming the importance of some residues for DENV-4 activity. The ASP121 residue appears to be the most important of the catalytic triad followed by TYR107 and ASP113, due to the large hydrophobic area exhibited in the two docking.With the validation of these two docking, it can be noticed that curcumin presents more reliable data for a possible drug candidate for the treatment of denguefever.

Keywords: Aedes aegypti. Curcuminoids. Flaviviridae. Molecular Docking.Antiviral.

1 INTRODUCTION

Aedes aegypti, a vector of dengue fever, is a viral disease transmitted to humans by a mosquito-borne mosquito from Egypt, Africa, dating back to the 16th century, which has spread throughout the world's tropical and subtropical regions. In 1762, it was described scientifically for the first time called *Culex aegypti*, but the description of the genus *Aedes aegypti* was only definitive in 1818. Currently, the dengue epidemic has been considered a major impact on public health.

Studies conducted at the University of Oxford (2013) in Britain, the disease reaches about 390 million people per year in the world, of which more than 90 million of these cases are presented in a serious manner and the remaining, mild or asymptomatic [1];

Dengue virus (DEN-V) is classified as an arbovirus belonging to the Flaviviridae family, genus Flavivirus, an infectious disease caused by any of the four antigenically related serotypes identified as DEN-1, DEN-2, DEN-3 and DEN -4 [2]. Similar to other Flaviviruses, the mature DENV consists of a single-stranded RNA genome, approximately 11 kilobases (Kb) in length, coated with an icosahedral protein (capsid), encoding a single polyprotein, which after cleavage, Generates three structural proteins that integrate the mature virus: Capsid (C) (M) and envelope (E), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5)[3].

From the earliest civilizations man sought in nature resources for therapeutic purposes, both in the alleviation of pain and in the treatment of diseases, which the use of medicinal plants based entirely on accidental discoveries. In the last decades with the growth of the pharmaceutical industries, large companies started to invest in the production of allopathic drugs and in the marketing of these products [4]. To ally them with modern medicine, they sought to study ways to understand the

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mechanisms of action of natural compounds on the pharmacological use of active principles of plant origin, highlighting natural products in drug development [5].

The ligand Curcumin is a member of the curcuminoid compound family, obtained from the roots of the Curcuma longa (*Curcuma longa*) plant native to Indian culture. Its use has been registered for centuries, both in medicine and in cooking, with considerable pharmacological activities including anti-inflammatory, anti-carcinogenic and antioxidant action [6]. Its antioxidant action has been attributed to its hydroxyl and methoxy groups, several studies characterize the anti-inflammatory action of curcumin, adept at anti-bacterial, antiviral, antifungal and antitumor action [7].

Through molecular anchoring methodology the present study aims to evaluate the participation of the Curcumin ligand and dengue virus serotype four protein (DENV-4). Molecular docking or molecular anchoring consists of the coupling between a receptor (protein or a nucleic acid molecule) and a linker (small molecule or other protein), resulting in a receptor / linker complex, providing the ligands of higher affinity to the binding site, predicting the possible combinations from the use of genetic algorithms based on the chosen three-dimensional structure [4]. In this context, attempts are made to seek a better orientation and more stable conformation that allows the analyst to identify which of these is the most probable in the interaction between target ligand [8]. In this way, the aim of this work is to perform in silico analysis of the interactions between the DENV-4 protein (NS1) and the Curcumin and Monodimethylcurcumin linker through the computational simulation.

2METHODOLOGY

2.1. Computer Resources

For this work, ACD / Labs ChemSketch® and Arguslab® software were used, which are free of charge, based on the Windows 7 Ultimate 64 Bit Operating System with Intel® x64 Core ™ i5-5005U CPU @ 4.0 GHz,12 GB of RAM .

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

From the ChemIdplus repository[9] (https://chem.nlm.nih.gov/chemidplus/) the ligand(Curcumin and Monodimethylcurcumin). Then Curcumin and Monodimethylcurcuminmolecules was characterized using ChemSketch® Freeware license software belonging to the ACD / Labs package[10]. The crystalline structure of the DENV-4 ED3 mutant protein with L387I PDB code (5B1C) was obtained from the Protein Data Bank[12] (http://www.rcsb.org/pdb/home/home.do) repository, A database for 131485 biological macromolecular structures.

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 [11]; (QM-AM1) using the Hartree-Fock SCF (200 max.interactions, 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 Arguslab® software [11] Freeware license, in which were removed the residues (H2O and SO4) that could influence the satisfactory result of the coupling[8].The 5B1C protein was subjected to a correction process, which was removed the solvent and SO4 residues, then it was saved in "Mol2" and soon after the same process occurred saving it to "pdbqt" extension occurring therein Methodology for the preparation of ligands.

2. 5.Docking Molecular

After preparation of the DENV-4 ligand and protein, molecular docking was performed using the Arguslab® software [11]. Using the flexible ligand and the rigid protein.

3 RESULTS AND DISCUSSIONS

The choice and visualization of ligands was obtained in the ChemIDPlus system, which is a free access database for research, which provides the nomenclature and structures used to recognize chemicals cited in the National Library of Medicine (NLM) databases) Including the TOXNET ® system (ChemIDPlus, 2016). After that, the first molecular modeling took place, starting with the Ligand drawings, obtaining the 3D structures (fig.1) of Curcumin and Monodimethylcurcumin some specific properties (Table 1) through the ChemSketch® software.

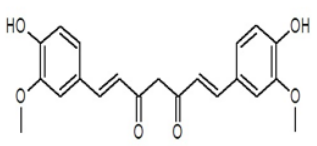
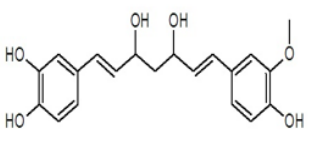
CURCURMIN		MONODIMETILCURCUMIN	
			
Molecular Formula	$C_{21}H_{20}O_6$	Molecular Formula	$C_{20}H_{22}O_6$
Formula Weight	368.3799	Formula Weight	358.38508
Composition	C (68.47%) H (5.47% O (26.06%)	Composition	C (67.03%) H (6.19% O (26.79%)
Molar Refractivity	104.04 ± 0.3 cm^3	Molar Refractivity	102.17 ± 0.3 cm^3
Molar Volume	287.8 ± 3.0 cm^3	Molar Volume	263.8 ± 3.0 cm^3
Density:	1.279 ± 0.06 g/cm^3	Density:	1.358 ± 0.06 g/cm^3

Fig.1. Structures of the ligands and some of their specific properties.

The structures are not always present a stable composition, causing distortions in molecules such as, lengths and angles of connections and dihedral angles presenting an unfavorable composition [13][14]. To correct these distortions, we used the processes of energy minimization and geometry optimization with of Arguslab® software. The calculation was based on quantum mechanics using the Austin Model 1 semi-empirical method (QM-AM1) (NDDO) using the Hartree-Fock (HF-SCF) open shell approach (UHF-Unrestricted Hartree-Fock), Configured for 300 interactions (1000 cycles), with a convergence value of 10-10 kcal mol-1 [15]. According to these parameters a stable energy formation was determined through the SFC interactions method, when analyzing the structures, the difference between the Ligands is identified, which the Ligand curcumin when submitted to the hydrolysis in alkaline medium loses one molecule of methanol.

Molecular docking is a computational technique performed through calculations that show the fit of two molecules in the three-dimensional space, thus seeking a preferred form with which a small ligand is accommodated in the active site of a Biological macromolecule and to estimate its binding affinity[16]. From the molecular anchoring methodology, Figure 3 demonstrates the molecular docking between the curcumin linker and the DENV-4 protein in Chimera® software.

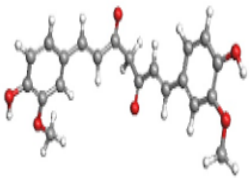
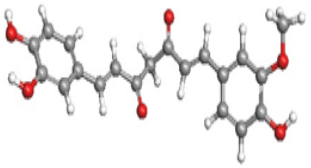
CURCUMIN	MONODIMETILCURCUMIN
	
Final Geom Energy = -179.4953135433 au	Final Geom Energy = -173.7890560366 au
Final Geom Energy=-11263.114 kcal.mol^{-1}	Final Geom Energy=-109054.3775 kcal.mol^{-1}

Fig.2. Three-dimensional structures of optimized ligands using the semi-empirical method.

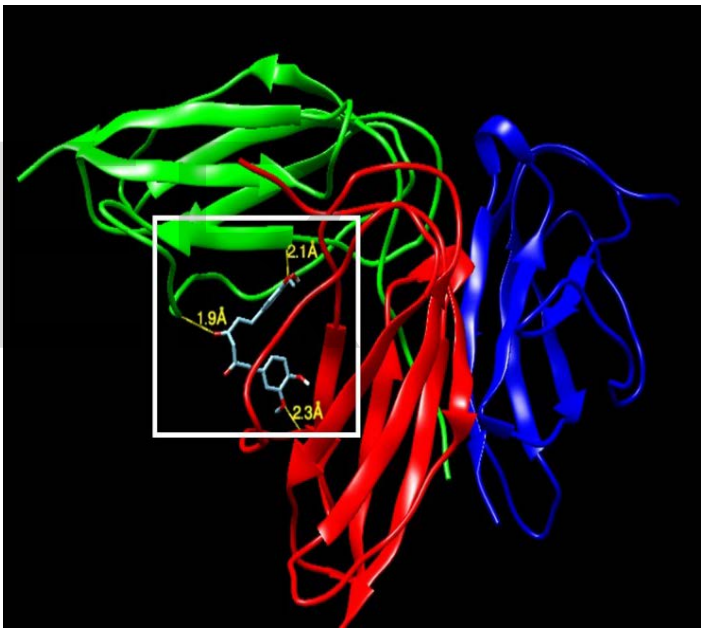


Fig.3. Molecular interaction between curcumin and DENV-4 as seen in CHIMERA® Software.

After the molecular docking between curcumin and DENV-4, 120 interactions between the ligand and the protein were verified. By the screening methods [4], an interaction showed 3 bonds in the protein with values of 1.9Å, 2.1Å, 2.3Å distance between O1-ASP121, O3-TYR107, O4-ASP113, respectively. The docking values of this interaction were 3.534Å (RMSD l.b.), 6.804 (RMSD u.b.) and Score of -5.9, in addition to 10 active torsions.Thus, as in the blind docking (Figure 3), all molecules interacted with the protein, however this time, they interacted specifically at the hydrophobic site, with a range of

energy ranging from -7.9 Kcal mol-1 to -10.4 Kcal mol-1. These energy values characterize a favorable interaction of the compounds with the protein, in which experimental analyzes could better clarify these results [8]. Figure 4 shows the interaction with the hydrophobic (red region) and hydrophilic regions (blue region) of the protein.

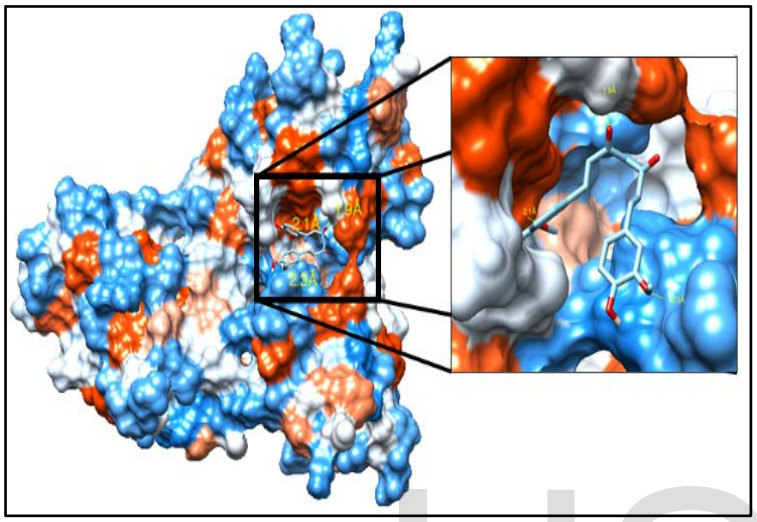


Fig.4. Active site between curcumin and protein DENV-4.

In figure 5 is shown the docking between Monodimethylcurcumin and DENV-4, in this docking was shown 89 interactions. The selected interaction presented RMSD l.b. Of 1.586Å, RMSD u.b. Of 5.766Å and a score of -6.5, Showed extremely strong O3 bonds with GLY152, ARG154 and VAL150 with distance values respectively of 2.2Å, 2.4Å and 2.8Å. According to figure 6, the interactions entailed a binding in the hydrophobic characteristic protein (red region) while the hydrophilic region did not look like bonds.

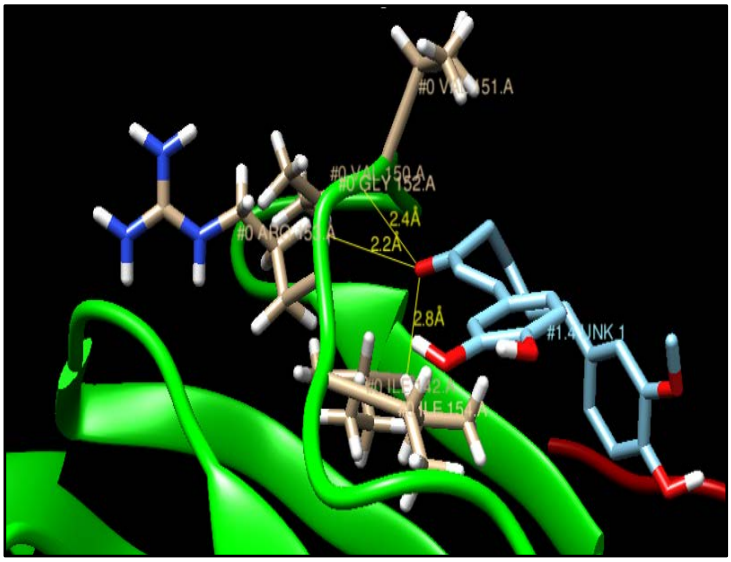


Fig.5. Molecular docking between Monodimethylcurcumin ligand and DENV-4 envelope protein, using UCSF Chimera® software.

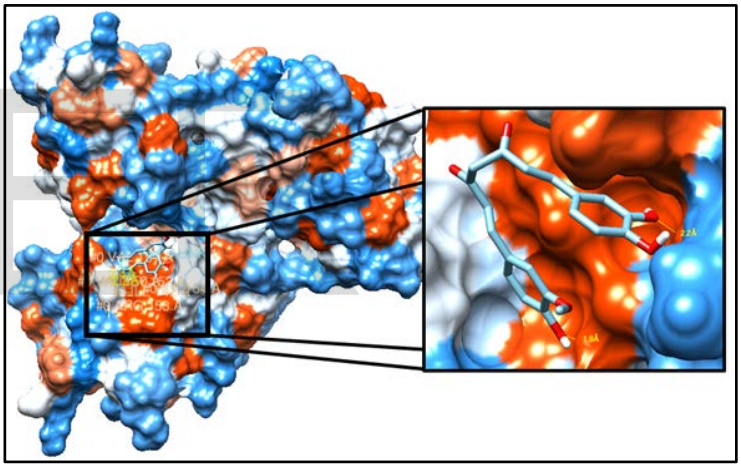


Fig.6. Interaction between Monodimethylcurcumin and DENV-4 protein (Active site), making use of the UCSF Chimera ® software.

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 [17]. 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 [18] [19].

4 CONCLUSIONS

The present work focused on the characterization and molecular docking studies using the Curcumin and Monodimethylcurcumin ligands and the 5B1C protein (PDB code) of the dengue virus, (DENV-4). In this perspective, the molecular docking results showed a correlation with the activity values of the compounds, confirming the importance of some residues for DENV-4 activity. The ASP121 residue appears to be the most important of the catalytic triad followed by TYR107 and ASP113, due to the large hydrophobic area exhibited in the two docking.With the validation of these two docking, it can be noticed that curcumin presents more reliable data for a possible drug candidate for the treatment of dengue, attacking by DENV-4, becoming a hope to produce new drugs.

That the bioinformatics software used in this research is of immense help in the production of new drugs to treating diseases, thus reducing the initial screening, allowing an optimization with respect to time and resources, and reducing the use of animals for Active principle recognition tests.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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