International Journal of Recent Research and Review, Vol. XII, Issue 2, June 2019

ISSN 2277 – 8322

In silico studies of the inhibitory potential of the phytochemical 3-Methylkaempferol present in Amburanacearenses (cumaru) on Apolipoprotein E4 (ApoE4)

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Abstract **– Alzheimer's disease (AD) is a neurodegenerative disorder whose cognitive and neuropsychiatric manifestation result in a progressive disability and eventual incapacitation in its patients, being considered as one of the social challenges and an important public health problem throughout the world. world, since it has no cure and no treatment, sufficiently effective to prevent its evolution. Amburanacearensis (CUMARU) is used as a medicinal plant, and its efficacy has been proven by means of pharmacological studies, being therefore used in the therapy of several diseases. In this context, the objective of this study was to evaluate the potential of the 3-Methylkaempferol phytochemical, present in Amburanacearensis as an inhibitor of Apolipoprotein E4 (ApoE4), through molecular docking simulations. 3-Methylkaempferol was optimized and prepared for Docking in the Arguslab® software configured to act with quantum (QM) and semi-empirical (QM-PM3) (NDDO) methods. Being the Docking realized in the software UCSF Chimera®. When analyzing the interactions of this ligand in the active site of ApoE4, evaluating the affinity and the specificity of the formed conformations, it presented favorable anchorages in the ApoE4 active site. In the most favorable conformation, the oxygen atom (O6) bound with the amino acid Glutamine (GLN) 135 of the A layer NE2 of the protein, obtaining a distance equal to 2.0 Å, which is the shortest binding distance of this coupling. Therefore, other short bonds of this same atom (O6) were found; in torsion # 1.3, it was observed that the oxygen atom (O6) bound with the amino acid Glycine (GLY) 106 of the HA2 layer of the protein, obtaining a distance of 2.3 Å, and with the Glycine (GLY) 106 of the layer AH, obtaining a distance of 2.8 Å. Thus, this ligand had good binding lengths and a function of stable scores, showing strong binding to the ApoE4 site, which showed that this ligand may come to develop therapeutic activity against Alzheimer's Disease, which can be ascertained and studied in future studies.**

Keywords **– Alzheimer, Molecular docking, Amburanacearensis.**

I INTRODUCTION

Alzheimer's disease (AD) or Alzheimer's disease has been considered as one of the most relevant social and health challenges in industrialized societies. Increased life expectancy and progressive aging facilitate the development of conditions associated with aging, such as the occurrence of pathologies. Thus, Alzheimer's disease is identified as one of the most prevalent dementia etiologies [1], corresponding to 60 to 70% of cases [2]. AD is defined as histopathologically by massive synaptic loss and neuronal death observed in the brain regions responsible for cognitive activities, including the cerebral cortex, entorhinal cortex, hippocampus and ventral striatum [3]. It is still characterized as a neurodegenerative and irreversible disease, which installs insidiously causing progressive decline of cognitive and motor functions [4], affecting mental functioning and eventual incapacitation in its patients [5-6].

As regards the development of this disease, it is known that polymorphisms in the apolipoprotein E (apoE) gene are important genetic risk factors for its development [7], being the main apolipoprotein found in the brain [3]. In humans, three major alleles of the apoE gene are found, resulting from only two DNA changes, called E2, E3 and E4. Therefore, other variants of apoE are identified from E1, E5 and E7, however, they are extremely rare [7]. Since, to date, numerous independent studies have shown that only the E4 allele of the apoE gene (19q13.2) has a reliable correlation with Alzheimer's disease [8]. As for their treatment, there is currently no curative treatment, only drugs that can improve the memory and behavior of the patient [8].

Amburanacearensis, a medicinal plant [9], has been widely used in folk medicine in the treatment of various diseases such as bronchitis, asthma, abdominal pain, rheumatism and muscle spasms [10].

Since it is widely used for therapeutic purposes, it has become indispensable for scientific studies to justify its use as a herbal remedy [11], and the effectiveness of the popular use of A. cearensis has been proven by studies pharmacological effects from the hydroalcoholic extract of the shell of the cauliflower and some of its chemical components, in which they showed analgesic, bronchodilator and anti-inflammatory activity [12], and studies have shown that the isozygous flavonoid has anti-inflammatory, antioxidant and bronchodilator, being pointed as one of the main active principles of the plant [13] and presents bioactive potassium against Parkinson's disease [14].

Since the chemically defined flavonoids as substances composed of three phenolic rings represent one of the most important and diversified groups of plant origin, they are generally found in leaves, flowers, roots and fruits of plants [2], exercising various activities such as: radiation protection UV, protection against microorganisms, enzymatic inhibition, antioxidant action, among others [15]. Since these biological and therapeutic activities are proven both in experimental conditions and in humans, what has made flavonoids an important object of study [16].

Thus, we sought to use Amburanacearensis as a study object and to identify one of the phytochemicals present in the stem of Amburanacearensis (Cumaru), potentially bioactive against neurodegenerative diseases, using the 3D structures of the potential therapeutic targets by the molecular docking method [14]. Since Molecular Docking is a modeling technique that allows the molecular combination process, between enzyme and inhibitor, where both molecules undergo conformational changes, occurring simultaneously to the interactions between them [17-18]. In the case of molecular docking, the complexes formed between protein-ligand and those possible complexes are analyzed in order to identify the most favorable ones, recognizing those with good steric and electrostatic complementarity between ligand-receptor, classifying the most potent compounds [4]. In this context, the objective of this work was to evaluate, through molecular docking simulations, the phytochemical potential of Ceurensis, 3-Methylkaempferol as an inhibitor of Apolipoprotein E4 (ApoE4).

II. METHODS AND MATERIALS

Initially, the structure of the bioactive component of A. cearensis, the binder: isocampferid, was obtained through the Chemspider repositories (http://www.chemspider.com/Chemical-Structure.4444394.html?rid=694c0a87-e61b-4a94-b554-6c1a3c741227) and obtaining the protein structure of Apolipoprotein E4 (ApoE4) domain 22k, a protein involved in Alzheimer's disease, used in Molecular Docking as a target molecule, obtained from the RCSB Protein Data Bank (http://www.rcsb.org). /pdb/home/home.do)[19], by X-ray diffraction, with the code PDB (1GS9) (https://www.rcsb.org/structure/1GS9) . Then, using the Arguslab® software [20] [21] configured to use the quantum (QM) and semi-empirical method PM3 (Parametric Method 3) (NDDO) [22] [23], with 200 interactions (1000 cycles) and with a convergence value of 10-10 kcal mol-1, the optimization of the geometry of the binder structure was performed, some of its electronic properties being obtained and analyzed, these parameters being important for the characterization of ligands [24] [25] [26]. Therefore, if using UCSF Chimera® [27], Molecular Docking with ApoE4 protein as target molecule and as a isocapferid ligand was performed, Docking calculations represent the central approach used in structure-based screening. This technique, developed / used to predict the best orientation and conformation of a binding molecule at its receptor site [28-30].

III. RESULTS AND DISCUSSIONS

The structure of the ligand 3-Methylkaempferol (5,7-dihydroxy-2- (4-hydroxyphenyl) -3-methoxy-4H-chromen-4-one) (FIG. 1), the main flavonoid found in Amburanacearensis, was obtained with the identification number ChemSpider ID: 4444394, some information being obtained from this compound as its molecular Formula C16H12O6; the percentage composition: C 64.00%, H 4.03%, O 31.97%; its average mass 300,263 Da and its monoisotopic mass: 300.063388 Da; its molar volume 190.0 ± 5.0 cm3 and density equal to 1.58 ± 0.1 g / cm3. After optimizing its structure, a final SFC energy equal to -88570.9702 kcalmol-1 and formation heat (ΔHf) of -163.5468 kcalmol-1.

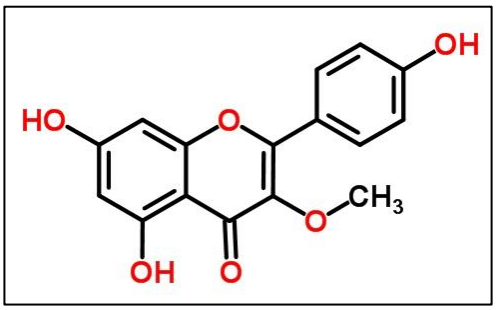

Fig. 1Molecular structure of 3-Methylkaempferol
Source: ChemSpider Repository
(http://www.chemspider.com/Chemical-Structure.4444394.html?rid=694c0a87-e61b-4a94-b554-6c1a3c741227)

The protein structure of the protein involved in neurodegenerative Alzheimer's disease, being Apolipoprotein E4 (ApoE4) (FIG. 2), was obtained in the RCSB Protein Data Bank repository by X-ray diffraction and with the PDB (1GS9) code, having a resolution equal to 1.7 Å, being classified as a binding lipid protein.

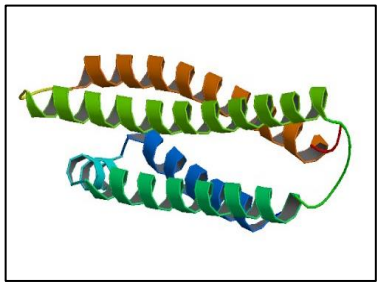

Fig.2 Protein structure of Apolipoprotein E4 (1GS9)

Source: RCSB Protein Data Bank. (https://www.rcsb.org/structure/1GS9)

In the Molecular Docking of Apolipoprotein E4 protein (ApoE4) with the 3-Methylkaempferol ligand, the target molecule (protein) was used rigidly, and the ligand flexibly, where it will seek the most stable / best orientation and in the receptor site of the protein. Molecular docking simulations use a conformational search algorithm [9], which predicts and analyzes the values of Free Binding Energy (ΔG), to locate the best positioning of the binder in the active site of the receptor. And the scoring function, which is used to describe the intensity of association or binding affinity between molecules (ligand-receptor), identifying and selecting the best formed conformations [31] [32]. With the results obtained in the molecular coupling between both (Table I), it was verified that the smaller bonding distance of this molecular coupling (FIG.. 3) was found in torsion # 1.6 whose binding energy value was -5.7 kcal mol -1 , which is used to describe the intensity / affinity of the association between the target molecule and the linker; already for the values of the root mean square deviation RMSD (2.228) indicates that this twist was presented as being the most stable.

Table I

Attractive Doctoral Twisting Between ApoE4 Protein and 3-Methylkaempferol Binder

Twists	**Bond energy(kcal mol -1)**	**RMSD**
#1.1	-6.3	0.0
#1.2	-6.3	27.391
#1.3	-5.9	4.603
#1.4	-5.8	17.195
#1.5	-5.7	37.971
#1.6	-5.7	2.228
#1.7	-5.6	35.923
#1.8	-5.6	4.446
#1.9	-5.5	26.899
#1.10	-5.4	18.072

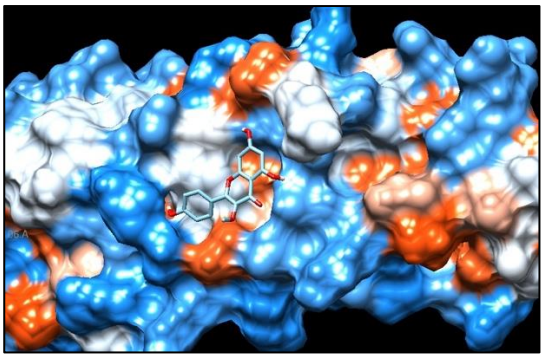

Fig. 3Molecular docking of the 3-Methylkaempferol ligand at the site of the ApoE4 target molecule

In this conformation the oxygen atom (O6) bound with the amino acid Glutamine (GLN) 135 of the NE2 A layer of the protein, obtaining a distance equal to 2.0 Å, which is the shortest binding distance of this coupling (Fig.. 4).

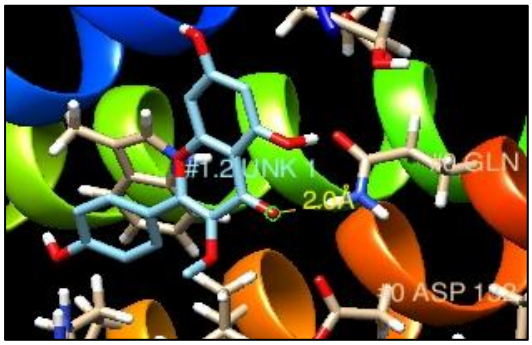

Fig.4 Linking distance of the ApoE4 Protein Molecular Coupling with 3-Methylkaempferol

As soon as other short bonds were found on this same atom (O6), and also with others, as for example on torsion # 1.3, it was observed that the oxygen atom (O6) bound with the amino acid Glycine (GLY) 106 of the layer The HA2 of the protein, obtaining a distance of 2.3 Å (fig. 5), and with the Glycine (GLY) 106 of the AH layer, obtaining a distance of 2.8 Å (fig. 6).

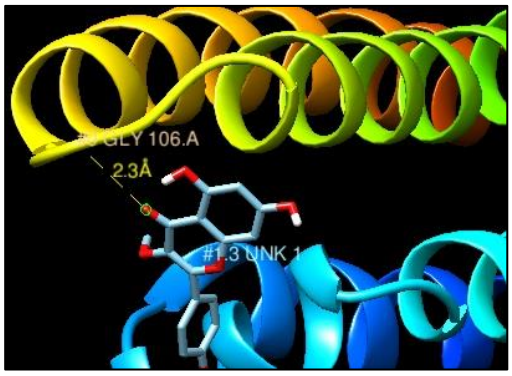

Fig.5 Linking distance of the ApoE4 Protein Molecular Coupling with 3-Methylkaempferol

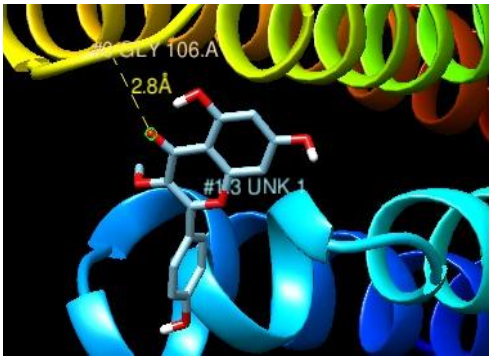

Fig.6 Linking distance of the ApoE4 Protein Molecular Coupling with 3-Methylkaempferol

IV. CONCLUSIONS

Molecular docking allows to analyze the interaction between a ligand at a macromolecular target, evaluating the affinity and the specificity and the conformations formed in the anchorage between protein-ligand. In this way the bioactive compound 3-Methylkaempferol presented favorable anchorages in the active site of the target protein, since, when analyzed the interactions between both molecules, this ligand had the lowest binding lengths and function of stable scores, showing strongly binding to the site of ApoE4, indicating that it is a promising inhibitory drug against this Alzheimer's disease-active protein.

V. ACKNOWLEDGMENT

The State University of Ceará (PROPGPQ / UECE) for the support. This work was partially supported by the Cearense Foundation for Science and Technology Support (FUNCAP), the Coordination for the Improvement of Higher Education Personnel (CAPES).

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