International Journal of Recent Research and Review, Vol. XI, Issue 3, September 2018
ISSN 2277 – 8322

Phytochemicals Present Inamburana Cearensis (Cumaru) Potentially Active Against Parkinson's Disease: A Docking Molecular Study

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Abstract **- Parkinson's disease (PD) is characterized as chronic, neurological and progressive disease, which causes the degeneration of neuronal cells causing problems in the motor part of the disease carriers, thus reducing the physical capacity and quality of life of the individual. Therefore, it has been observed that in the popular medicine the use of herbal products has been increasing, and that the therapeutic activities of these products have been confirmed scientifically. Thus, Amburanacearensis (Cumaru) has been widely used and its efficacy has been proven by pharmacological studies, and its chemical components have shown many therapeutic activities. In this perspective, the present work had as objective to identify and characterize the three main active principles of this plant: coumarin, isocampferídio and amburosídio (a), and to use them like ligands in Docking Molecular, in order to verify the affinity of these ligands in the active site of the Parkine protein (PARK2), which is important for the development of PD. Therefore, some of the electronic properties of the ligands were analyzed using Arguslab® software configured to act with quantum (QM) and semi-empirical (QM-PM3) (NDDO) methods. And with Autodock vina® and UCSF Chimera® Molecular Docking simulations were performed. With the results obtained in the Docking, a good interaction of both ligands was observed in the active site of this PARK2 target molecule. In the linkage conformations, as well as in the formed bonds, coumarin showed a good interaction, where in the coupling twist # 1.4, the scoring function score was -5.2 and the roots: RMSD lb 19.595 Å and RMSD ub 20,835 Å; the oxygen atom (O1) bound with the amino acid (GLY) 178 of the A chain of the protein, obtaining a binding length of 2.0 Å; and the oxygen atom (O2) was ligated with the (ALA) 179 of the A chain, whose bond length was 2.2 Å and with (GLY) 178, having binding length of 2.5 Å, smaller coupling distances and more favorable linkage.**

Keywords **- Amburosídio (a). Coumarin.Isocampferídio. PARK2.Theoretical Chemistry.**

I. INTRODUCTION

Currently Brazil has undergone changes of epidemiological profile, in which, the population is undergoing an aging. Thus, with the increase in the elderly population, the number of chronic diseases associated with aging, such as Parkinson's disease (PD), is defined as a primary degenerative disease localized in the substantia nigra compact, in which it is synthesized to dopamine [1].

This chronic, neurological, progressive disorder affects the motor part of the carrier, since the degeneration in basal ganglia cells causes a loss or interference in the action of dopamine, which is the main neurotransmitter of the basal ganglia, and they cooperate for the accuracy and the uniformity of the movements and coordinate the changes of position [2]. Thus, the degenerations of these neuronal cells end up causing slowness, tremors, muscular rigidity, changes in posture and gait, and difficulties in speech and writing, which reduces the individual's physical capacity and quality of life [3]. As it is characterized as chronic disease, it has no intervals or periods of relief of symptoms, manifesting progressive and severe effects that cause suffering, wear and increasing tension in its bearer [4].

Regarding the development of this disease, a protein that is related to one of the forms of family

PD has been identified, which is called Parkine (Parkin), in which it is encoded by the PARK2 gene [5]. Since parkin is a ubiquitin E3 ligase protein, a constituent of the ubiquitin system, which is an important mechanism of degradation of adenosine triphosphate-dependent protein [6], acting as a regulator of protein degradation, and having a essential role for cellular mitochondrial integrity [7].

On the other hand, it has been observed that in the last years, a world-wide interest has grown significantly, for herbal products, being these, medicinal products obtained from medicinal plants [8]. In this wide range of phytotherapics, Amburanacearensis (Cumaru) [9] stands out. Due to the great use of Amburanacearensis, in popular medicine, research and testing have been carried out, and chemical and pharmacological studies have allowed to characterize cumaru, in order to define the active principles, such as coumarin and ambushid A, and its pharmacological potentials [10]. The efficacy of the popular use of A. cearensis has been confirmed by pharmacological studies on the hydroalcoholic extract of the bark of the cauliflower and some of its chemical components, in which it has shown therapeutic activity [9], [11].

Due to its great use and efficacy in the use of folk medicine, Amburanacearensis was used as an object of study to identify and characterize electronics, structurally and energetically, 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. Since Molecular Docking aims to find the most probable and stable affinity and orientation of a ligand, anchored in the active site of a target receptor [12] [13], it is possible to identify subsequently which compounds linked to the given receptor, and to classify which of these compounds had higher affinity at this active site [14] 15].

II. METHODOLOGY

Initially, the main active principles present in the stem of *Amburanacearensis*(Cumaru) were identified, and the two - dimensional structure of these bioactive components were obtained. These structures were obtained through a virtual Screening using the Chemspider repositories (http: //www.chemspider. with /)and Pubchem (https://pubchem.ncbi.nlm.nih.gov/).

Soon after, the three-dimensional structure of the protein involved in Parkinson's disease was investigated and the Parkine protein (PARK2) was used as the target molecule. Since there are structures obtained in the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) repository, in the form of PDB (Protein Data Bank) files obtained by X-ray diffraction, by of the PDB code (4BM9).

Soon after, simulations were performed and the geometries of the molecular structures were optimized. As the best classically optimized structures were used as a starting point for a new series of geometric optimizations using semi-empirical quantum methods, different Hamiltonians were used for comparative purposes (AM1, PM3, PM6) and obtaining the UV / Visible (ZINC). In this work, the Arguslab® software [16] was configured to operate with quantum (QM) and semi-empirical (QM-PM3) (NDDO) methods, where it was analyzed some of its electronic properties as well as its electrostatic potential. , with 200 interactions (1000 cycles) and with a convergence value of 10-10 kcal mol-1, to perform the optimization of the geometry. Given that these parameters can / should be validated through calculations performed using the DFT.

All the software used for simulation are free access, based on the Windows 7 Ultimate 64-bit Operating System with Intel® Core ™ i3-5005U CPU @ 2.0 GHz processor, 4GB of RAM and 1000GB of HD. If using Autodock vina® and UCSF Chimera® [17]. To perform the Molecular Docking, Docking calculations represent the central approach used in structure-based sorting. These techniques are designed to predict the best orientation and conformation of a binding molecule at its receptor site.

III. RESULTS AND DISCUSSIONS

Molecular docking was performed initially to obtain the molecular structures of the ligands. These are the main active principles present in Amburanacearensis

(Cumaru): coumarin, isocampferid and Amburoside (a). Soon after, they were using semi-empirical quantum methods, to perform simulations and to optimize the geometries of molecular structures. Thus, the first ligand to be obtained was coumarin (Figure 1) which is the main chemical component found in the plant. Then, through the Chemspider repository (http://www.chemspider.com/), the molecular structure of this ligand was found, through the identification number ChemSpider ID: 13848793, through the repository it was possible to obtain its molecular formula C9H6O2, the value of its average mass: 146,143 Da and its monoisotopic mass: 146.036774 Da. Coumarin, formally known as: 2H-Chromen-2-one according to IUPAC, has percentage composition: C 73.97%, H 4.14% , O 21.90%; molar volume of 117.0 ± 3.0 cm3; surface tension of 46.4 ± 3.0 dyne / cm and density of 1,248 ± 0.06 g / cm3. These data were obtained through the characterization of this ligand. After optimizing its molecular geometry, a stable energy conformation was obtained by the method of interactions SFC -40245.7282 kcal mol-1 and heat of formation (ΔHf) -37.9636 kcalmol-1 calculated by the atomization energy, using the enthalpies of atoms of atomization.

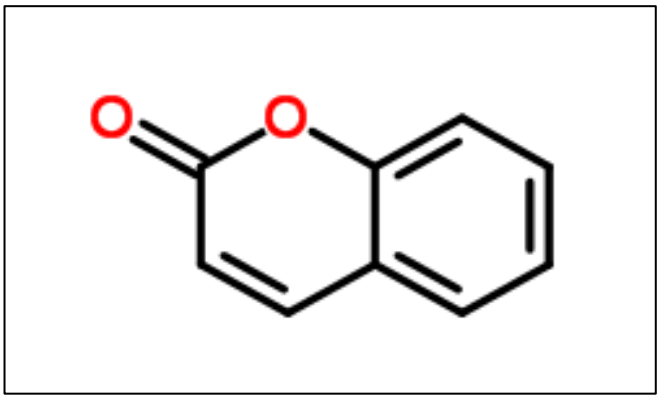

Fig.1. Cumarina molecular structure
Source: Repositório ChemSpider, 2018.
(http://www.chemspider.com/ChemicalStructure.13848793.html?rid=468ab177-b808-49f5-ada0-b06f2d47bf37)

Then, the molecular structure of the second ligand used in the Molecular Docking test was obtained, being this Isocampferídeo (Figure 2) the main flavonoid found in Amburanacearensis. Its structure was obtained through the Chemspider repository, and was found by the identification number ChemSpider ID: 4444394. This ligand according to IUPAC is formally known as: 5,7-dihydroxy-2- (4-hydroxyphenyl) -3-methoxy-4H-chromen-4-one, having as molecular formula: C 16 H 12 O 6; percentage composition: C 64.00%, H 4.03%, O 31.97%; average mass 300,263 Da; monoisotopic mass: 300.063388 Da; 190.0 ± 5.0 cm3 molar volume; and density equal to 1.58 ± 0.1 g / cm 3. After optimizing the structure of this ligand, a final SFC energy of -88570.9702 kcalmol-1 and formation heat (ΔHf) of -163.5468 kcalmol-1 was found.

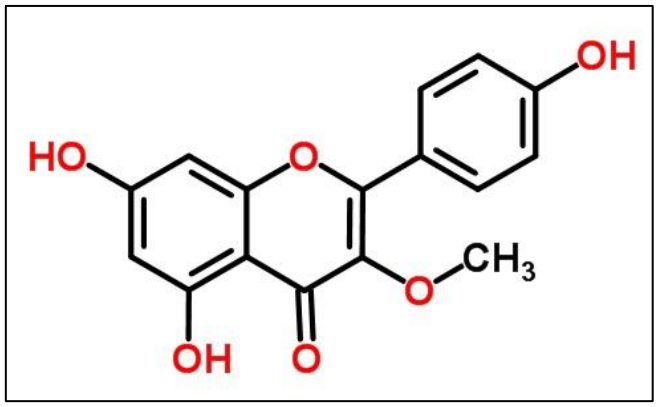

Fig.2. Isocampferid molecular structure
Source: Repositório ChemSpider, 2018.
(http://www.chemspider.com/ChemicalStructure.4444394.html)

The third ligand obtained and used in the Molecular Docking tests was Amburoside (a) (Figure 3), a phenolic glycoside found in A. cearensis. This was obtained through the Pubchem repository, with the following identification register: CID: 10409977. It has the molecular formula: C20H22O10 and its molecular weight equal to 422,386 gmol-1, and according to IUPAC its nomenclature is: [3,4 [4 - [(2S, 3R, 4S, 5S, 6R) -3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] oxyphenyl] methyl dihydroxybenzoate. For this ligand was also obtained some important properties, such as its percentage composition: C 56.87%, H 5.25%, O 37.88%; molar volume of 271.9 ± 3.0 cm3; surface tension of 76.4 ± 3.0 dyne / cm and density of 1.553 ± 0.06 g / cm3, these data being obtained through the characterization of thisligand.

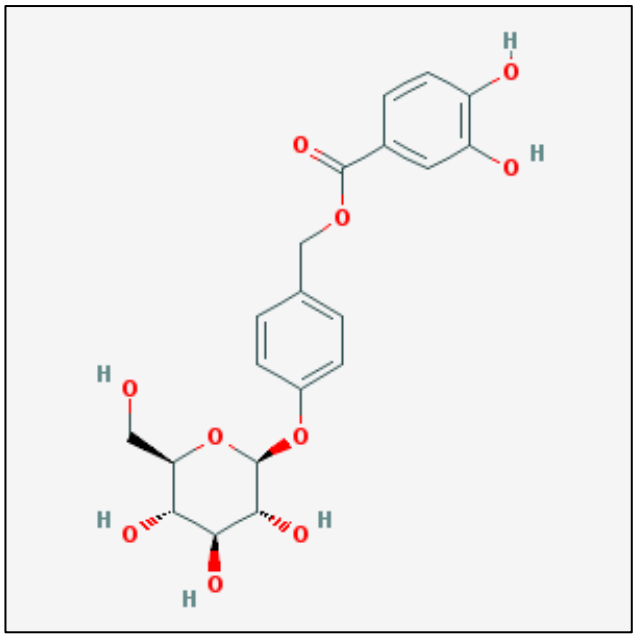

Fig.3. Molecular structure of the Amburoside (a)
Source: Repositório Pubchem, 2018.
https://pubchem.ncbi.nlm.nih.gov/compound/10409977

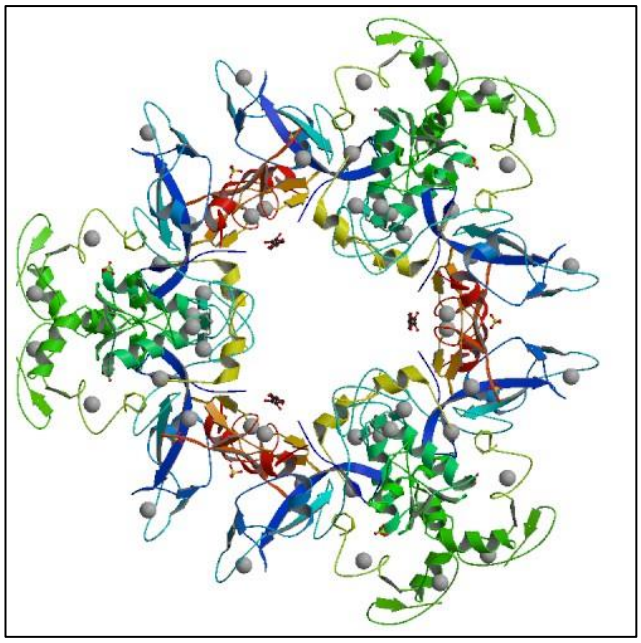

Fig.4. Protein structure of PARK2 (4BM9)
Source: RSCB Protein Data Bank,2018.
https://www.rcsb.org/structure/4BM9

Then, the protein structure of the protein involved in the neurodegenerative Parkinson's disease was obtained, with the protein used as target molecules being the Parkine protein (PARK2) (Figure 4). This structure was obtained through the RCSB Protein Data Bank repository (http: /www.rcsb.org/pdb/home/home.do) by X-ray diffraction, it is registered with the PDB code (4BM9), having a resolution of 2.25 Å, being classified as a ligase.

Thus, the first molecular anchorage test was carried out, using as a target molecule the protein Parkine (PARK2) and as a coumarin ligand. In this study, the region where the coupling, also called the "induced fit" between coumarin and the active site of the target molecule, was observed, with attractive twisting between them (Table I). It can be observed that the lowest distances of this coupling (Figure 5) were found in the torsion was # 1.4, having a value of -5.2 of the score function and a root mean square deviation RMSD: RMSD lb 19,595 Å and RMSD ub 20,835 Å.

Table I
Attractive dozing twists between the Parkine protein (PARK2) and the coumarin

CHIMERA MODEL	SCORE	RMSD L.B	RMSD U.B
#1.1	**-5.7**	**0.0**	**0.0**
#1.2	**-5.6**	**1.21**	**2.612**
#1.3	**-5.3**	**24.915**	**26.333**
#1.4	**-5.2**	**19.595**	**20.835**
#1.5	**-5.2**	**17.378**	**17.802**
#1.6	**-5.1**	**20.725**	**21.391**
#1.7	**-5.1**	**24.903**	**26.373**
#1.8	**-5.0**	**14.174**	**15.289**
#1.9	**-5.0**	**1.805**	**2.111**
#1.10	**-4.9**	**15.958**	**17.068**

Source:*dados da pesquisa*.

Where the oxygen atom (O1) bound with the amino acid Glycine (GLY) 178 of the A H chain of the protein, obtaining a binding length of 2.0 Å; and with the amino acid Tyrosine (TYR) 177 of the A H chain of the protein, obtaining a binding distance of 2.7 Å. In this same torsion, the oxygen atom (O2) was ligated with the alanine (ALA) 179 of the A chain, whose bond length was 2.2 Å and Glycine (GLY) 178, having a bond length of 2, 5 Å. Already at torsion # 1.5 the oxygen atom (O2) bound with Arginine (ARG) 130 A HH12 chain whose binding distance was 2.1 Å and Arginine (ARG) 130 at the location of the chain A HH22 , whose distance was 2.2 Å. These are the smallest distances in the molecular coupling of coumarin in the active site of the Parkine protein (PARK2) and the most stable attachment position (Figure 6).

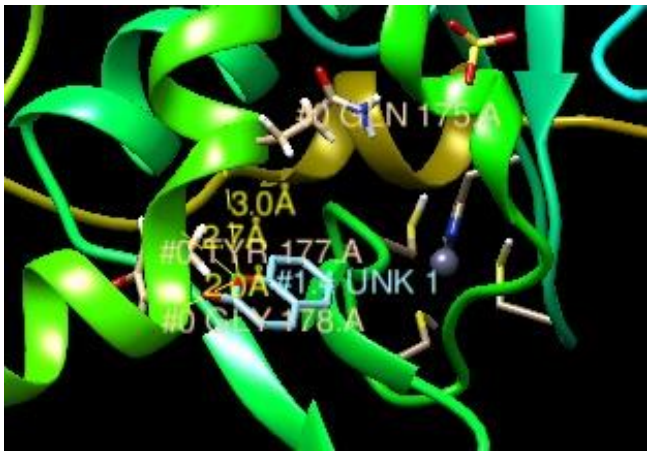

Fig.5. Linking distance of the molecular coupling of PARK2 Protein (4BM9) with coumarin.

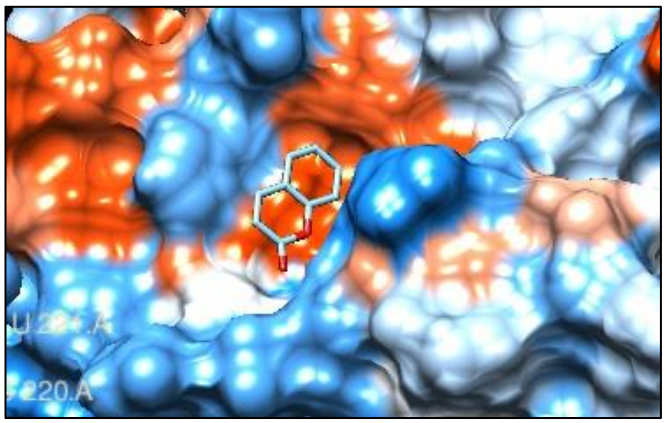

Fig.6. Molecular coupling of the coumarin linker at the active site of the target molecule PARK2 (4BM9).

With the molecular anchoring of the Parkine protein (PARK2) with the Isocampferid ligand, the coupling between the two was obtained, and it was possible to identify where the Isocampferid ligand interacted with the active site of the target molecule (PARK2), where orientation and conformation occurred more favorable and stable between them, through the data obtained (Table II). In the anchoring torsion # 1.3, which for score function has a value of -6.5 and the root mean square deviation values RMSD: RMSD l.b 31.738 Å and RMSD u.b. 35.12 Å, where the lowest binding distances were observed (Figure 7).

Table II

Attractive Twins of the Docencia between the protein Parkine (PARK2) and the Isocampferídeo

CHIMERA MODEL	SCORE	RMSD L.B	RMSD U.B
#1.1	**-7.1**	**0.0**	**0.0**
#1.2	**-6.5**	**22.176**	**24.207**
#1.3	**-6.5**	**31.738**	**35.12**
#1.4	**-6.5**	**31.532**	**34.675**
#1.5	**-6.3**	**31.774**	**34.988**
#1.6	**-6.3**	**35.696**	**37.688**
#1.7	**-6.3**	**30.213**	**33.014**
#1.8	**-6.2**	**32.764**	**35.293**
#1.9	**-6.2**	**14.169**	**18.021**
#1.10	**-6.2**	**31.496**	**35.294**

The oxygen atom (O3) was observed to bind: with the Threonine (THR) 129 A HG1 chain whose binding length was 2.2 Å; with Arginine (ARG) 130 A HH12 chain, whose binding length was 2.7Å; and with the HG21 A chain Threonine (THR) 129, with binding length of 2.8Å. And that the oxygen atom (O5) was bound with Aspartate (ASP) 238, obtaining a bond distance of 2.1Å, these being some of the shorter bond distances. In this twist, the molecular coupling of Isocampferid in the PARK2 protein (4BM9) (Figure 8), was more favorable.

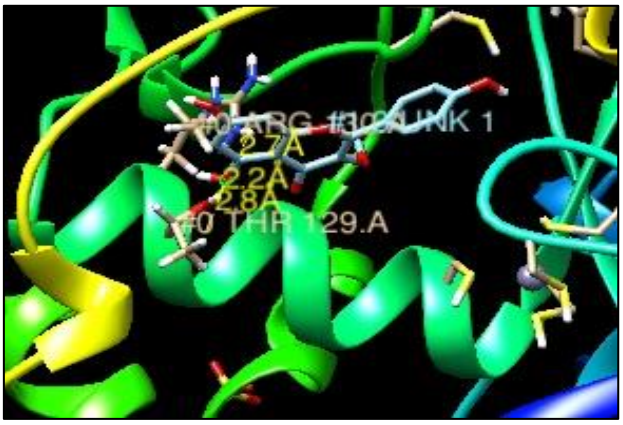

Fig.7. Linking distance of the molecular coupling of PARK2 Protein (4BM9) with Isocampferide.

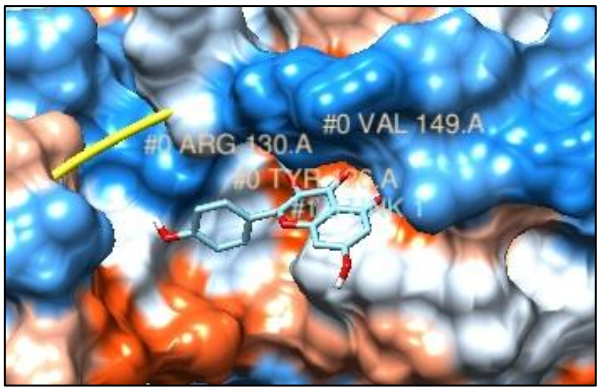

Fig.8. Isocampferidligand molecular coupling in the active site of the target molecule PARK2 (4BM9).

Therefore, with the Molecular Docking between the Parkine protein (PARK2) and the Amburosidio(a) ligand , the coupling of this ligand was obtained at the active site of this target molecule, and the best conformations formed could be identified and selected (Table III) where some of the shortest link-length distances between protein-ligand (Figure 9) were selected. Some of these connections were found in torsion # 1.2, having a value of the function of scores of -7.2, and 18.148 Å and 21.055 Å, for the values of root mean square deviation RMSD: RMSD lb and RMSD ub, respectively.

Table III

Attractive Twins of the Docing between the Protein Parkine (PARK2) and the ligandAmburosídeo (a)

CHIMERA MODEL	SCORE	RMSD L.B	RMSD U.B
#1.1	**-7.7**	**0.0**	**0.0**
#1.2	**-7.2**	**18.148**	**21.055**
#1.3	**-7.2**	**23.019**	**26.146**
#1.4	**-7.1**	**16.141**	**21.23**
#1.5	**-7.1**	**20.027**	**22.339**
#1.6	**-7.0**	**21.379**	**25.727**
#1.7	**-7.0**	**19.549**	**23.005**
#1.8	**-7.0**	**25.204**	**27.432**
#1.9	**-6.9**	**19.406**	**23.065**
#1.10	**-6.9**	**21.264**	**23.658**

In this torsion the oxygen atom (O9) bound to Asparagine (ASN) 49 of the A H chain of the protein, obtaining a binding distance of 2.0 Å; to Prolin (PRO) 48 from A HÁ chain, whose distance was 2.5 Å; to the Glutamate (GLU) 66 A chain, obtaining a bond distance of 2.6 Å; and the A chain alanine (ALA) 65 of the protein, yielding a binding length of 2.7 Å. It is possible to observe the "induced adjustment", that is, the coupling between this ligand and the active site of the most favorable target molecule (Figure 10).

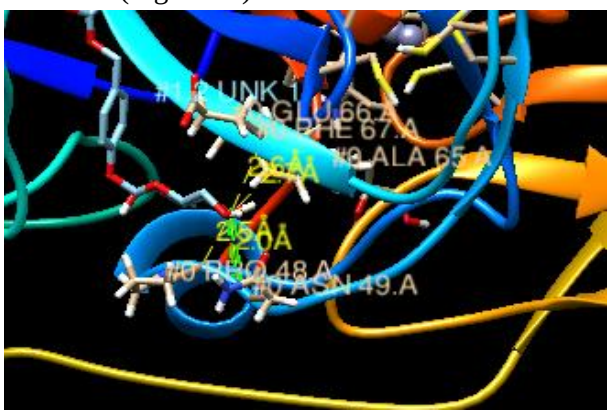

Fig.9. Linking distance of the molecular coupling of PARK2 Protein (4BM9) with Amburoside (a).

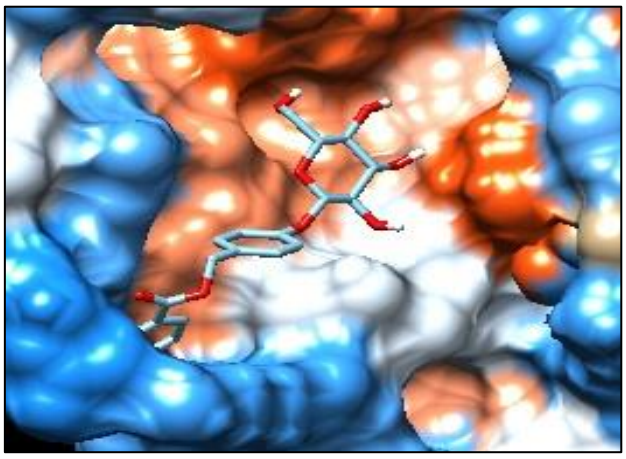

Fig.10. Molecular coup ling of the ligandAmburoside (a) in the active site of the target molecule PARK2 (4BM9)

IV. CONCLUSIONS

The three main active principles found in *AmburanaCearensis* (CUMARU): coumarin, isocampferid and Amburoside (a), submitted to semi-empirical calculations (QM-PM3) (NDDO) of the software, electronically characterized, converged and optimized , and used as ligands in Molecular Docking simulations have been shown to be propitious and may have activity against or inhibitory to Parkin protein (PARK2), which may be used as active compounds against Parkinson's disease (PD) in the future. Since in the Dock tests with the Parkin protein (PARK2), both ligands were complexed in the protein with favorable coupling twists, and the ligand which most stood out as to the results of its molecular coupling in the active site of PARK2 was coumarin ligand. Since, when analyzed the fitting conformations, as well as the bonds formed between them, coumarin showed a good interaction at the PARK2 receptor site, the smallest distances of this coupling being found in the torsion was at 1.4, having this is a value of -5.2 of the scoring function, where the oxygen atom (O1) bound with the amino acid Glycine (GLY) 178 of the HA chain of the protein, obtaining a binding length of 2.0 Å; and with the amino acid Tyrosine (TYR) 177 of the A H chain of the protein, obtaining a binding distance of 2.7 Å. At this same torsion the oxygen atom (O2) bound with the alanine (ALA) 179 of the A chain, whose bond length was 2.2 Å. Already at torsion # 1.5 the oxygen atom (O2) bound with Arginine (ARG) 130 A HH12 chain whose binding distance was 2.1 Å and Arginine (ARG) 130 at the location of the chain A HH22 , whose distance was 2.2 Å, presenting as the most stable ligand-protein complex. Since this ligand has therapeutic properties, such as anti-inflammatory, anticoagulant, antioxidant, antitumor and espamolytic activity, which validates the use of this plant in popular medicine.

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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