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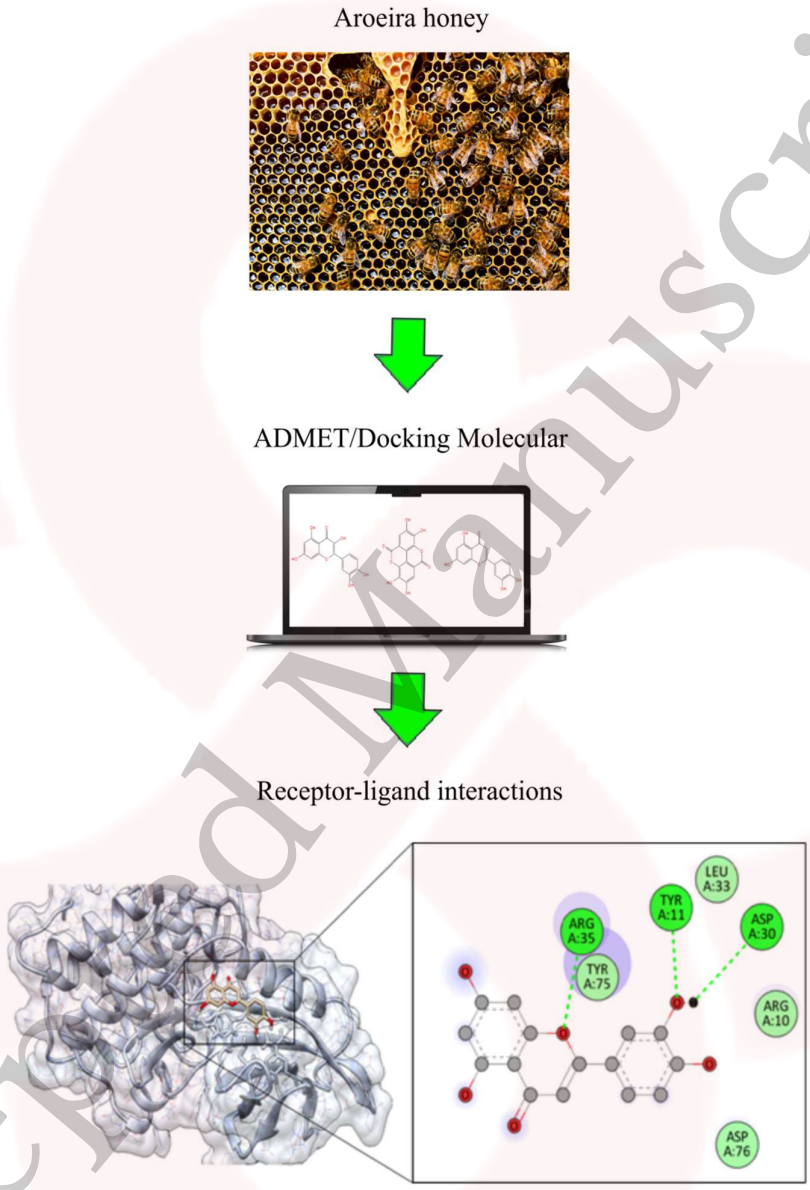

In Silico Investigation of the Constituents of Aroeira Honey (*Astronium urundeuva*) and the Binding Affinity with Important Proteins of *M. leprae* and *M. tuberculosis*

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Abstract

The use of natural products has been gaining a lot of attention in recent years, among them organic honey, with its high phenolic content, has great potential in fighting diseases. The Aroeira-do-Sertão (*Astronium urundeuva*) is a tree that, when it blooms, is pollinated by bees and produces a honey rich in nutrients that is widely used by the population to treat flu and other symptoms. Leprosy and tuberculosis are two of the main neglected diseases that occur frequently in underdeveloped countries, where a large part of the population is vulnerable to contagion and treatment is often ineffective. Such diseases can cause several symptoms such as motor loss and major respiratory complications in patients. Recent research shows a great interest of the pharmaceutical industry in the production of medicines and in the fight against these diseases, opening a great opportunity for studies to present satisfactory results. The present study seeks to carry out an in-silico investigation of the main constituents of Aroeira honey, performing pharmacokinetic predictions through ADME-Tox platforms. From molecular docking, simulations were performed with the proteins ML2640c, PknA and PknB, in which the ligands Quercetin (-8.8 kcal/mol), Ellagic acid (-8.3 kcal/mol) and Luteolin (-8.1 kcal/mol) present the best binding affinities with important amino acid residues of the catalytic site. In addition, anticarcinogenic, anti-inflammatory and antibacterial activities were predicted for these compounds, but more in-depth studies, such as in vitro and in vivo assays, are necessary to prove the biological activity of these molecules.

Keywords: Molecular Docking; Neglected Diseases; Aroeira Honey; Phytodrugs.

1. Introduction

Neglected Tropical Diseases mostly occur in the underdeveloped countries with no or bad initial sanitation system that lead to causing these diseases. There is a range of neglected diseases, such as Leprosy, Tuberculosis, Leishmaniasis, Chagas Disease, Dengue, etc., that are not treated by agencies in the countries where these are established, which eventually could lead to become a serious problem, increasing the number of infections of these diseases in the poorest populations [1].

Mycobacteria are bacillary organisms that cause infectious diseases through skin contact with the bacteria which are known to cause deadly diseases and are a major threat to human health.[2]. The range of illnesses caused by these bacilli is diverse, spanning from simple bacterial infections to more severe conditions such as leprosy and tuberculosis. Both are deadly, extremely aggressive and can take a long time to present symptoms. This delayed onset of symptoms creates a significant challenge for early diagnosis, as the diseases often go undetected until they manifest clinically [3, 4, 5, 6].

Leprosy is a disease that attacks the nerves in the skin and Schwann cells in the tissue, causing motor loss in the region, as well as the appearance of light or dark spots in the region where the bacillus attacks [7]. However, Hansen's disease has several clinical forms in addition to the classic symptoms and, without exception, all forms are equally lethal to the human being affected by the disease. [7, 8, 9, 10]. Each clinical form has distinct symptoms, with Tuberculoid Leprosy (TH), Indeterminate Leprosy (IH), Virchowian Leprosy (VH), and Borderline Leprosy (DB) being the main types of clinical forms. Each clinical form of Leprosy has several symptoms, which vary between aggressive neural lesions, neurological alterations, skin lesions, loss of motor sensitivity, internal atrophy of lesions, erythematous or hypochromic areas of skin, nodules, papules and macules, as well as lesions of the nerve trunks [11, 12, 13, 14].

According to reports from the World Health Organization (WHO), leprosy is present in a total of 182 countries belonging to the territories administered by the WHO, including African countries, the Americas, the Eastern Mediterranean, Europe, Southeast Asia and the Western Pacific. In 2022, a total of 174,087 new cases of leprosy were detected in the world, with 71.44% of these cases (124,377) concentrated in Southeast Asia, mainly in India, considered the country with the highest cases of leprosy in the world [15].

Tuberculosis is a disease caused by infection with the bacillus *Mycobacterium tuberculosis*, which has been affecting humanity since the earliest civilizations. According to [16, 17, 18], tuberculosis is a disease that attacks the lungs and causes several symptoms, including coughing, expectoration, weakness, moderate chest pain, irritability, weight loss and night sweats. Furthermore, when tuberculosis is combined with other diseases, it increases the number of deaths and the treatment loses its effect, which is the case for people infected with HIV (Human Immunodeficiency Virus), where tuberculosis becomes much worse, leading to death in a short time [19, 20].

Again, WHO reports very high numbers of tuberculosis cases around the world, mainly concentrated in countries such as India, China, the Philippines, and African countries, presenting more than 2.000.000 cases of tuberculosis in 2022, showing a great concern regarding the future of the disease [21].

Both diseases are mentioned in several studies, such as [22], which mentions the complexity of treating diseases quickly and affordably around the world. This has led to the emergence of phytopharmaceuticals. Phytopharmaceuticals are drugs produced through natural extracts of plants, fruits, honey, and teas, where the compounds present in each product are systematically analyzed to indicate the medicinal potential of the specific compound [23-30].

Among medicinal products, honey has been widely discussed in studies, such as [31, 32, 33], which point out the medicinal potential of the compounds in several of the most complex symptoms to determine the potential for action against fatal diseases. Recent researches, such as [34, 35, 36], points out the medicinal potential of Aroeira honey based on the chemical composition found in the honey. According to [37, 38, 39, 40, 41], there is a high phenolic load and several medicinal compounds, in addition to vitamins essential for the metabolic functions of the human body. This has sparked great interest in research on Aroeira honey, determining the compounds and delimiting its medicinal use.

Therefore, the use of specialized platforms to perform pharmacokinetic and pharmacodynamic predictions is present in silico studies to determine how the compounds act with the various ADME-Tox parameters (Absorption, Distribution, Metabolism, Excretion, and Toxicity) used within the platforms [42, 43, 44].

These parameters are quite significant since they indicate and forecast the behavior of a specific compound in the body. They determine which proteins and cells the compound binds to, or which biochemical reactions it interferes with, how the compound is metabolized, and whether it might harm the body [42, 43]. These tests open a range of research opportunities, and precisely the opportunity for the emergence of medicines based on natural compounds found in various local products, such as honey, molasses and syrups, from Aroeira honey or other active ingredients derived from various natural products found in all regions of Brazil and the world [45].

However, ADME-Tox tools are essential for defining the pharmacological properties of compounds and for categorizing them as usable or unusable. Not only are ADME-Tox tools essential, but the use of computational simulations is also of great help regarding the direct interactions of compounds [46, 47, 48]. Techniques that incorporate the Molecular Docking approach allow the direct engagement of compounds with the principal proteins present in the bacilli. They perform docking in computational environment, thus predict Binding Energy values of how tightly the ligand is entrenched in the protein binding site and what type of chemical interactions the compound has with all the amino acid residues of the proteins [49, 50, 51].

Recently, computer simulation research has used Molecular Docking as one of the main methods and infectious diseases as the main target, to demonstrate how a compound behaves in the face of the disease [52]. This type of simulation reduces exorbitant expenses in experimental research preparations; after all, the simulations are constantly improved, contributing to having a direction and, in the future, the works to be used as a basis for experimental research [52, 53, 54].

This technique is based on a series of parameters that are already established and that are constantly updated, both in computer science, cheminformatics, bioinformatics, and the pharmaceutical industry, which in turn uses it in the production of medicines, proving their use or even in the development of research with medicinal of natural origin [55].

With this in mind, *M. leprae* is a bacterium that has a complex cell wall rich in fatty acids and peptidoglycan synthesis as well as proteins that play crucial roles in the virulence of the bacillus. In the eyes of the docking methodology and according to the studies of [56], the proteins that play a certain role in the virulence of the bacterium are possible docking targets. The ML2640c protein is a protein whose biological function has not been fully cataloged by biochemical experimental assays. However, according to the studies of [57], this protein has S-adenosylmethionine (AdoMet) binding centers, which has the function to aid in the transfer of methyl groups (CH3) to the substrate, playing an extreme biological function of the bacterium. By inhibiting this protein, some functions that help the bacillus proliferate in the host, as well as the health of the bacteria, can have their effects reduced or inhibited, which causes complications in its health and the virulence potential of the bacillus, making it a great target to be explored.

Accordingly, *M. tuberculosis* proteins should be chosen for their potential to become targets for experimental assays. Therefore, proteins such as PknA and PknB are excellent targets due to their diverse metabolic functions for bacterial growth and their extreme importance for several physiological processes of the bacteria [58, 59]. This makesthem a great target to be explored and attacked by the bioactive compounds of Aroeira honey. By using the proteins, it is expected that their biological functions will be affected and damaged in such a way as to interfere with the health of the bacillus, placing the Aroeira compounds on a new level.

The use of silico technologies gives possibilities in the identification of disease targets such as Leishmaniasis, Tuberculosis, Leprosist, Dengue, etc. These methods rely on computational studies such as molecular docking, molecular screening, molecular modeling and molecular dynamic. They contain crucial advice for drug synthesis or the employment of investigated proteases and suggest effective solutions in combating several neglected diseases and new strategies to develop effective drugs to prevent the transmission of these diseases [60, 61, 62, 63].

Therefore, the main objective of this research is to investigate, in silico, the main ADME-Tox values, as well as to determine the binding energy values and chemical interactions of the main compounds from Aroeira honey.

2. *Materials and Methods*

2.1 3D Structure of ligands and receptors

The crystallographic structure of the *M. leprae* target protein was obtained from the Protein Data Bank [57] under (PDB code: 2UYO), along with the *M. tuberculosis* targets (PDB code: 4OW8 and 1MRU) [58, 59]. However, the three-dimensional structures of the Aroeira honey ligands were obtained from the NCBI PubChem website [64].

2.2 Obtaining cavities

The cavities of the investigated proteins were obtained through the CavityPlus platform. Within the platform, the respective PDB codes of the targets are loaded, and the platform tracks, based on the residues, and the cavities, as well as showing the format, druggability values, and their respective coordinates [65].

2.3 Molecular Docking

The structures were prepared using the Chimera software [66], where the receptor structure was loaded to remove any residues that might be averse to the structure as well as to remove ligands, if any. Docking was performed using the Autodock Vina software [67], with the aid of Autodock Tools [68]. Autodock Tools was used to perform the previous optimizations to adapt the receptor structure for ligand anchoring. First, the receptor is loaded, then nonpolar hydrogen atoms are added to the structure, followed by a Gasteiger charge. The structure is saved as the system macromolecule in pdbqt format.

Next, the Grid Box information was added, considering the studies by [52] that determine a size on the x, y, z axes equal to 22 Å, with a number of modes equal to 50 and a length of 1 Å. Then, the coordinates were added based on the CavityPlus results, being for *M. leprae* (x -32.75, y 1.0, z 6.25) and *M. tuberculosis* (x -8.75, y 7.0, z -21.0 and x 1.25, y 26.25, z 12.0), respectively. After that, the ligand was loaded, and the torsion tree was verified and saved in pdbqt format. Docking in Autodock Vina in the operating system Command Prompt continued.

Upon completion of docking, an _out file is generated where only the ligand with the lowest binding energy should be used. With this, the complex was assembled and opened in the Discovery Studio Visualizer 2024 software [69], where the chemical interactions of the complex were denoted and verified.

ADMET predictions

The ADME-Tox properties of the ligands were obtained by submitting the structures (3D, 2D, or SMILES code) to the ADME-Tox platforms. For this study, we used the SWISSADME [42] PREADME [43], and Way2Drug (PASSONLINE) [70] platforms.

The parameters analyzed were Aqueous Solubility, Rule of Five, Human Intestinal Absorption (HIA), Blood-Brain Barrier (BBB), permeability in Caco2 cells and MDCK cells, P-glycoprotein (P-gp), AMES Test (TA100 and TA1535), Carcinogenicity in rats and mice and biological assays [71-79].

3. *Results and Discussion*

3.1 Compounds present in Aroeira-do-Sertão honey

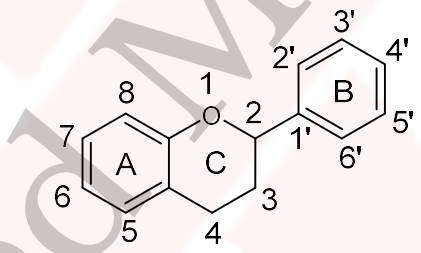
Aroeira honey is characterized by its dark color and exotic flavor, which is attractive to the consumer's palate. In addition to being widely consumed by local people, Aroeira honey is the subject of medicinal studies that correlate the chemical constituents of honey with medicinal treatments and the production of phytopharmaceuticals [33, 34, 35, 36, 37].

Studies on the physical-chemical characterization of Aroeira honey shows a high phenolic load among the compounds, in addition to B vitamins [80], derived from acids of natural origin and minerals such as Iron, Magnesium, and Calcium [81]. During the research, several compounds were found in Aroeira honey, among them the most used in several natural products, such as flavonoids, Quercetin, Luteolin, Ellagic Acid, Fisetin, Rutin and other compounds classified in Aroeira honey. To avoid doubts, all the compounds found are indicated in Supplementary Table **1S**, where the large presence of phenolic compounds, acids, and other natural substances found in Aroeira honey is notable.

Recent and old studies indicate a great phytotherapeutic potential of flavonoids in combating various symptoms, including potential antioxidant, anti-inflammatory, and, most importantly for the purpose of this research, antibacterial uses. Studies such as [82, 83, 84, 85, 86] promote a large collection of flavonoids that act on bacterial diseases to reduce the proliferation of bacteria in the body.

These phytotherapeutic actions of flavonoids are due to the molecular structure of the compounds, and the structures are similar, sharing the same basic skeleton, differing only in the functional groups, and this is exactly what delimits the phytotherapeutic potential of flavonoids. The presence of isomers among flavonoids is very common. Their structure is represented in Figure **1**.

Figure **1:** Flavonoids basic structure.



Font: Chandra, Panche, Diwan, 2016.

Observing its structure in Figure 1, it is evident that the functional groups can be arranged in various ways. According to the studies of [82, 83], the functional groups that link together give rise to several other compounds and classes of flavonoids, as well as different medicinal properties. For example, flavonols exhibit specific characteristics when hydroxyl groups are present in the aromatic ring, C5-C8, and/or in C1'-C6', in addition to a ketone at C4 and a double bond forming a pyrene at C2-C3. Also, the different forms of organization of the functional groups lead to new compounds, giving rise to flavones, containing only alcohol (-OH) as a functional group arranged in different carbons in the structure. The different constitutional organizations of the basic skeleton also lead to new compounds with different properties, making it justifiable that different functional groups lead to different medicinal properties.

3.2 ADME-Tox Predictions

During the ADME-tox tests, two sets of predictions were performed, the first of which was performed to delimit, based on key predictions, which compounds, if isolated, end up causing

harm to the body. Among the parameters used, some key parameters were considered (BBB, Rule of Five, P-gp, Carcino Mouse, and Carcino Rat) to isolate the compounds and reduce the number of compounds to be predicted and used in the Docking simulations.

Supplementary Table **2S** shows the values and parameters used to isolate the compounds. Under no circumstances should the compounds cross the blood-brain barrier. If this happens, the compound could cause damage to the Central Nervous System (CNS), the brain, and damage adjacent to the CNS [87]. As is the case with the compound Abscisic Acid where it crosses the barrier, causing possible damage to the CNS. Compounds that cross the Blood-Brain Barrier or are positive in the rodent groups (rat and mouse) are discarded. However, compounds that show alternating positivity in the rodent groups are kept, which is the case with some of the compounds, such as Quercetin, which showed alternating positivity in the rodent groups.

Among the compounds in Supplementary Table **2S**, it is preferable that they present normal to high intestinal absorption, but this does not reflect in the classification of the compounds, it only dictates how quickly the compound is absorbed in the stomach.

The Rule of 5 denotes certain parameters that candidates must fulfill to be classified: a molecular weight of no more than 500 g/mol, an n-octanol water partition coefficient (Log $P_{O/W}$) of no more than 5, hydrogen donors of no more than 5, and hydrogen bond acceptors of no more than 10 [88]. For compounds that do not respect the Rule of 5, they have been eliminated, even if only a few aspects do not fit the rule, as is the case with Myricetin, which passed the parameters of BBB and Carcino Rat and Mouse but presented a violation of the Rule of 5 (NhorH>5). The same reasoning for Chlorogenic Acid, where it respects the parameters of BBB and Carcino Rat and Mouse but a violation of the Rule of 5 (NhorH>5). This does not mean that the compound is unusable; it was just established that the compounds that did not fully respect the Rule of 5 were not selected for the next stages of investigation. These parameters were checked in SWISSADME.

By isolating the compounds that respected the parameters defined in the first set of predictions, the second set of predictions is carried out, where new parameters that determine the pharmacokinetic and pharmacodynamic part of the candidate compounds are considered. Therefore, Supplementary Table **3S** shows the separated compounds as well as their predicted values.

Analyzing Supplementary Table **3S**, the first point analyzed is the solubility values. For this study, Log S (ESOL) was used which relates the solubility of the compound. According to [89] the values to be analyzed cannot be less than -6.00 and greater than 0.00. For a better understanding of the Log S (ESOL) value, the aqueous solubility potential is denoted right after, where the closer to zero, the greater the solubility of the compound. Almost all the compounds proved to be within the parameters recommended by [89], However, one of the compounds, Succinic Acid, showed a value of 0, indicating a high solubility potential, as did Caffeic Acid (-1.89).

Caco2 and MDCK cells are very important cells when it comes to administration, compound absorption, delivery to different areas of the human body, and drug design. All in all, the compounds should not obtain values greater than 70 nm/s for Caco2 cells. It is preferable for them to have only medium permeability, with values between 4-70 nm/s. However, studies such as that by [90, 91] indicate that high permeabilities have a positive impact on phytopharmaceutical studies. Only a few of the compounds proved to be contrary to the values

for Caco2, falling below the recommended level, with Quercetin (3.412), Quercetin 3,3'-dimethyl ether (2.996), Baicalein (1.280), Catechin (0.656), Epicatechin (0.656), and Pinobanksin (3.691) being the compounds that fell below the recommended level.

MDCK cells are of great help in the transportation of compounds through the body, so medium values are necessary in order not to cause damage to the respective cells. According to the studies of [90], the values to be considered are between 25-500, which delimits medium permeability in the cells. Once again, some compounds exhibited values that were contrary to the recommended levels, with two of them being significantly lower: Succinic Acid (8.511) and Rosmarinic Acid (0.202), respectively.

The AMES Salmonella test is carried out on strains of the *Salmonella typhimurium* species to determine the chemical and genetic mutation potential of the candidates. We determined two strains, TA100 and TA1535. Compounds that test positive indicate a high potential for chemical and biological mutations in the compounds. It is preferable for candidates not to be positive, but studies such as that of [78] indicate that compounds, even if they are positive in the tests, are usable in biological tests, and small mutations can be made to the structure of the compound so as not to harm the guinea pigs. For the AMES test, all the compounds showed mutagenicity. For the AMES Salmonella test, the compounds should not show mutagenicity in both groups (TA100 and TA1535), as is the case with Quercetin, Kaempferol, Kaempferide, Isoharminetin, Luteolin, Baicalein, Hesperetin, Eridictyol, Fisetin, Catechin and Epicatechin, which were negative in the TA100_10RLI strains and both the TA1535_10RLI and TA1535_NA strains, but positive in the TA100_NA strain. Of all the compounds, only one showed negativity in all the TA100 and TA1535 strains, Lumichrome.

Human Intestinal Absorption (HIA) is a parameter that determines how well a compound can be absorbed by the human intestine. It is preferable for compounds to have values greater than 70, indicating a high potential for intestinal absorption. Values below 70 indicate that the compound may take a while to be absorbed by the intestine [73]. Some compounds were below the recommended values, such as Succinic Acid (54.538), Quercetin (63.485), Rosmarinic Acid (62.487), Gallic Acid (53.696), Ellagic Acid (61.395), Catechin (66.707) and Epicatechin (66.707).

3.3 Biological Prediction

The biological predictions were performed on the PASSONLINE platform, where the most recurrent biological activities were searched for in articles such as [70]. Among the various results of PASSONLINE, the potential for anti-inflammatory, antioxidant, antibacterial, anticarcinogenic, and healing actions was analyzed.

Among the results, it is preferable that they are above 0.500 for activity potentials (*pa*); however, activity values greater than inactivity values (*pi*) still report an activity potential, even if low. The values can be seen in Table **1**.

Table **1:** Biological activity values of the best compounds in Aroeira honey.

Compounds	Anti-inflammatory		Antioxidant		Antibacterial		Anticarcinogenic		Healing	
	Pa	*Pi*	*Pa*	*Pi*	*Pa*	*Pi*	*Pa*	*Pi*	*Pa*	*Pi*

Caffeic Acid	0.651	0.023	0.603	0.005	0.358	0.041	0.571	0.014	0.225	0.094
Vanillic Acid	0.505	0.055	0.374	0.014	0.376	0.036	0.413	0.029	0.267	0.067
Quercetin	0.689	0.017	0.872	0.003	0.387	0.033	0.757	0.003		
Quercetin 3,3'-dimethyl ether	0.707	0.015	0.777	0.004	0.985	0.034	0.778	0.006		
Quercetin 7,3'-dimethyl ether	0.710	0.014	0.754	0.004	0.384	0.034	0.746	0.007		
Apigenin	0.644	0.024	0.732	0.004	0.391	0.032	0.641	0.011	0.256	0.073
Kaempferol	0.676	0.019	0.856	0.003	0.395	0.031	0.715	0.008	0.174	0.151
Kaempiferide	0.627	0.026	0.765	0.004	0.365	0.039	0.733	0.008		
Isoharmetin	0.663	0.021	0.809	0.003	0.375	0.037	0.779	0.006		
Genistein	0.626	0.027	0.765	0.004	0.394	0.031	0.715	0.004	0.191	0.128
Luteolin	0.661	0.021	0.775	0.004	0.388	0.033	0.690	0.009		
Baicalein	0.674	0.019	0.801	0.003	0.399	0.030	0.610	0.012	0.182	0.140
Hesperetin	0.640	0.024	0.746	0.004	0.370	0.038	0.783	0.006		
Narigenin	0.660	0.021	0.794	0.003	0.397	0.031	0.724	0.008		
Eriodictiol	0.691	0.017	0.817	0.003	0.395	0.031	0.775	0.006		
Gentisic acid	0.716	0.014	0.406	0.012	0.419	0.026	0.409	0.029	0.305	0.045
Rosmarinic acid	0.453	0.072	0.539	0.005	0.222	0.100	0.398	0.031	0.183	0.139
Lumicrome							0.252	0.082	0.166	0.163
Sinapic Acid	0.612	0.029	0.576	0.005	0.359	0.041	0.616	0.012	0.179	0.144
Saringic Acid	0.498	0.058	0.403	0.012	0.395	0.031	0.413	0.029	0.252	0.076
Gallic Acid	0.548	0.044	0.520	0.006	0.418	0.026	0.395	0.031	0.276	0.061
Ellagic Acid	0.749	0.010	0.699	0.004	0.380	0.035	0.396	0.031		
Protocatechuic acid	0.538	0.046	0.401	0.012	0.394	0.032	0.387	0.033	0.302	0.047
Fisetin	0.642	0.024	0.787	0.004	0.346	0.044	0.687	0.009		
Galangin	0.689	0.017	0.853	0.003	0.393	0.032	0.703	0.009	0.176	0.148
Catechin	0.548	0.044	0.810	0.003	0.320	0.053	0.795	0.005		
Epicatechin	0.548	0.044	0.810	0.003	0.320	0.053	0.795	0.005		
Pinobanksin	0.712	0.014	0.940	0.002	0.381	0.035	0.780	0.006		
Dl-*p*-hydroxyphenyllact acid			0.285	0.026	0.201	0.117	0.382	0.034	0.195	0.123

Font: Way2Drug (PASSONLINE), 2024.

It is noteworthy that the compounds vary in their values; not all compounds exhibit high activity in the selected areas, but many demonstrate strong activity in other areas, as shown in Table 1. As is notable for the Isoharmnetin compound, which showed an excellent value for antioxidant activities, but which showed no healing activity or a very low activity, it was not possible to obtain its values. The same can be said for Fisetin, which has interesting anti-inflammatory,

antioxidant, antibacterial and anticarcinogenic values, but has no value for healing potential. This shows the great complexity of Aroeira honey and the great bioactivity of Aroeira honey.

3.4 Molecular docking

After selecting the ligands, the Molecular Docking simulations began. Docking was performed on the three bacterial proteins using the coordinates established by CavityPlus, and the results of the Binding Energies of the ML2640c, PknA and PknB protein can be seen in Table **2**.

Table **2:** Binding Energy values from Molecular Docking simulations with the respective targets.

Ligands	**Receptors PDB ID**		
	2UYO	4OW8	1MRU
	Kcal/mol	Kcal/mol	Kcal/mol
Quercetin	-8.8	-8.0	-7.7
Fisetin	-8.8	-7.7	-7.4
Luteolin	-8.6	-7.7	-8.1
Baicalein	-8.4	-7.4	-7.8
Eriodictiol	-8.4	-7.7	-7.9
Hesperetin	-8.3	-7.8	-7.7
Catechin	-8.3	-7.7	-7.5
Epicatechin	-8.2	-7.2	-7.7
Apigenin	-8.2	-7.6	-7.8
Rosarinic	-8.2	-7.5	-8.0
Kaempferol	-8.2	-7.7	-7.5
Narigenin	-8.2	-7.5	-7.6
Ellagic acid	-8.1	-8.3	-8.1
Isoharminetin	-8.1	-7.6	-8.1
Quercetin 3,3'-dimethyl ether	-8.1	-7.2	-7.9
Lumichrome	-8.0	-7.5	-7.5
Quercetin 7,3'-dimethyl ether	-8.0	-7.6	-7.2
Pinobanquisin	-7.9	-7.6	-7.3
Genistein	-7.8	-7.1	-7.2
Kaempferide	-7.8	-7.7	-7.5
Galangin	-7.7	-7.7	-7.5
Caffeic Acid	-6.5	-6.0	-6.1
Dl-*p*-hydroxyphenyllact acid	-6.5	-6.1	-5.4
Gallic acid	-6.2	-5.5	-5.3
Sinapic Acid	-6.1	-5.8	-5.7
Gentisic acid	-6.0	-5.1	-5.1
Protocatechuic acid	-5.9	-5.6	-5.3
Syringic acid	-5.9	-5.5	-5.3
Vanillic	-5.7	-5.5	-5.2

acid

Font: AutoDock Vina, 2024.

It is noteworthy that most of the ligands obtained good binding energies, which according to studies by [52] determine that values below -7.0 are ideal for the assembly and analysis of the complexes. Among all the dockings worked on for all the protein targets, the complex was assembled for only two ligands of each target worked on.

Therefore, the binding energies for 2UYO (Quercetin and Fisetin) are exceptionally low (-8.8 and -8.8) suggesting very good anchoring. This low binding energy affects the chemical accessibility of the ligands in relation to the protein residues since their entrapment based on van der Waals forces ensures better direct interaction and ligand stability considering the frequent motions and chemical reactions of the protein. Moreover, an increase in the size of the ligand is also observed altering the binding energy value; therefore, large ligands interact with more residues than the small ligand. For 4OW8 (Ellagic Acid and Quercetin) the same principle applies, where their resulting binding energy was -8.3 and -8.0 respectively, being optimal binding energy values, as well as the values for 1MRU (Luteolin and Isorhamnetin) where their respective binding energy values are -8.1 and -8.1, signaling that the ligands are perfectly anchored within the catalytic site.

After successful anchoring of the ligands in the protein, a series of chemical interactions between the amino acid residues are expected, where the interactions can be very diverse, depending, of course, on the atoms and their reactive nature of both the amino acid residue and the ligand. For the purpose of phytopharmaceutical design the interactions to be investigated are hydrophobic interactions and hydrogen bonds. As noted by the work of [88], these interactions are useful in shielding of the ligand from other fierce biological interactions with the protein. Furthermore, stated by [92] hydrogen bonds originate from static charge between bottom bonds from elements more electronegative than hydrogen primarily oxygen. These interactions are considered to be important because they weaken the cell walls of the protein, thereby creating entry point for the drugs. In certain cases, they may degrade the protein; they will not allow any physiological repair mechanism to occur; this leads to cell necrosis.

Not only are these interactions essential, but there are interactions that favor ligand anchoring, which are the van der Wals interactions [93]. Interactions that play a crucial role in the stability of the ligand within the cavity. The Pi-Cation interactions [93] and interactions between the pi electron-rich orbitals that help to keep the ligand less volatile, being the Amide-pi Stacked interactions, carried out by the overlap of amide groups. All these interactions are essential in drug design because they play crucial roles not only in protection but also in the stability and electrostatic attraction of the ligand to the cavity residues, contributing to a better binding energy [94]. However, the most favored interactions observed in the context of phytopharmaceuticals are observed in Table **3**.

Table **3:** Best interactions between complex ligand-protein.

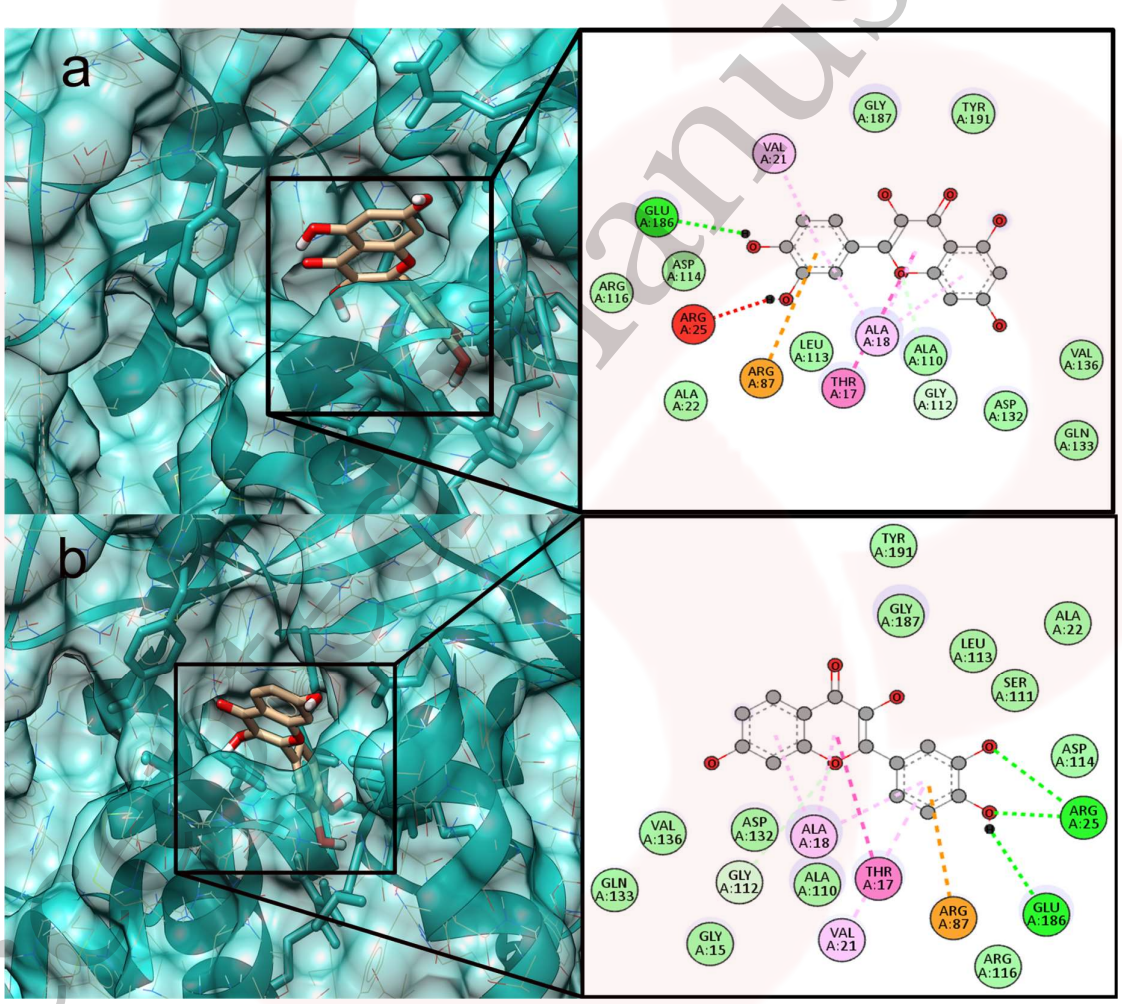
Ligands	Hydrogen Bonds	Pi-cation	Amide-pi Stacked	van der Wals
Quercetin	Glu A:186, Met A:178,	Arg A:87.	Thr A:17.	Arg A:116, Asp A:114,

	His A:192, Gln A:188, Asp A:193.			Gly A:187, Tyr A:191, Ala A:22, Leu A:113, Ala A: 110, Asp A:132, Gln A:133, Val A:136, Met A:176, Val A:171, Ala A:169, Gly A:191, Asp A:167, Arg A:140.
Fisetin	Arg, A:25, Glu A:186, Arg A:25.	Arg A:87.	Thr A:17.	Gln A: 133, Val A:136, Asp A:132, Ala A:110, Arg A:116, Tyr A:191, Gly A:187, Leu A:113, Ser A:111, Ala A: 22, Asp A:114.
Ellagic Acid	Gln A:188, Ala A:169, Gln A:188.			Asp A:167, Arg A:140, Ala A:168, Gly A:191, Pro A:170, Val A:171, Ala A:189, Ile A:184.
Luteolin	Arg A:35, Tyr A:11, Asp A:30.			Tyr A:75, Leu A:33, Arg A:10, Asp A:76.
Isoharminetin	Arg A:35.	Arg A:35.		Leu A:33, Val A:37, Asp A:76, Arg A:10.

Fonte: BIOVIA, 2024.

Note that not all the interactions are shown in the table, due of course to their limited medicinal use and scope. Some of the interactions are unfavorable for the permanence of the ligand, as can be seen in the interaction between Quercetin (**a**) and Arg A:25 in Figure **2**, which is reddish in color in the 2UYO protein.

Figure **2:** Maps of interactions with the amino acid residues of the catalytic site of the ML2640c protein (PDB ID: 2UYO). (**a**) Quercetin and (**b**) Fisetin.



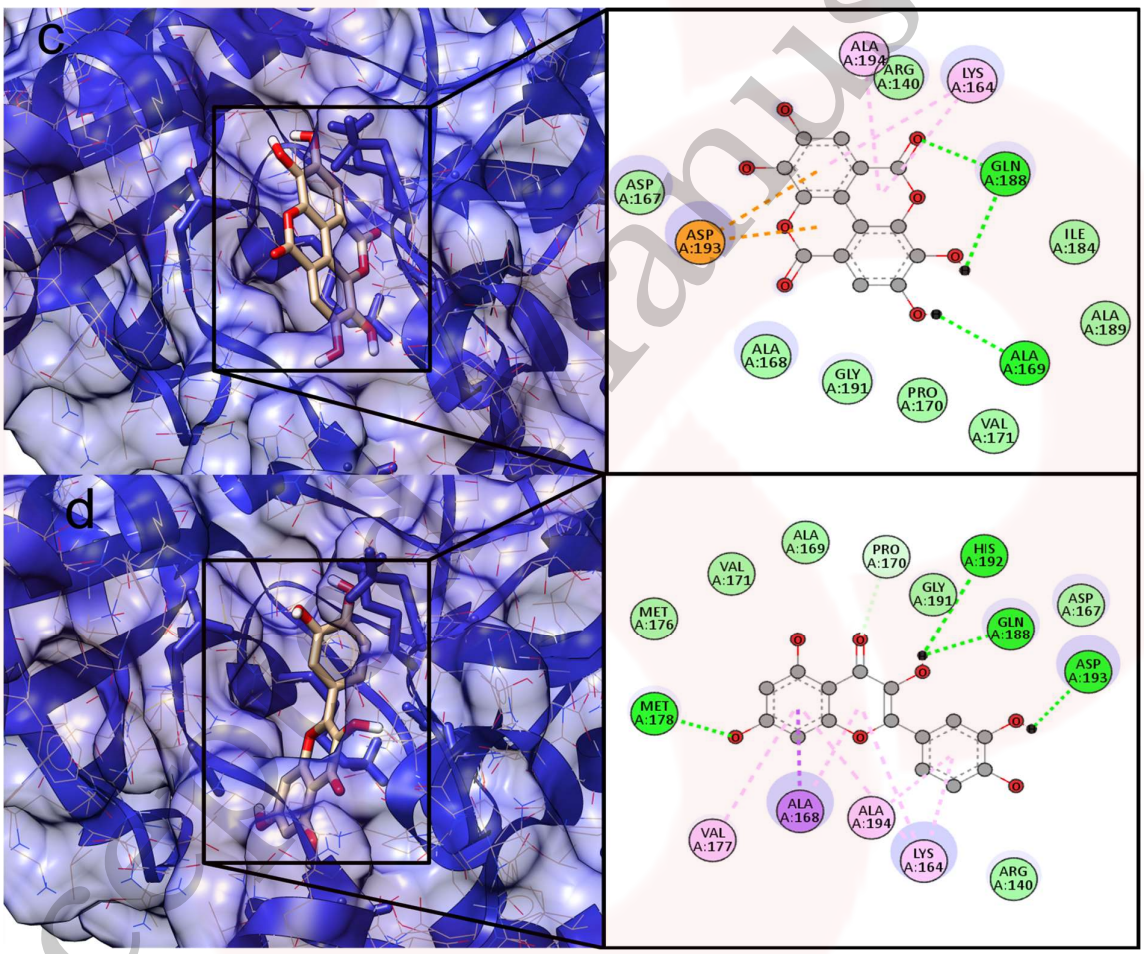
Font: BIOVIA, 2024.

This interaction is called Donnor-Donnor and plays a role in repelling the ligand at the hydrogen of the C3' hydroxyl, and the principle of like repels like applies to this interaction. However, only one Donnor-Donnor interaction is observed in just one of the complexes. Of all the interactions observed in Figure **2**, only one covalent interaction between carbon and hydrogen is observed in both complexes, present at residue Gly A: 112. This interaction doesn't affect

anything in the complex, just a classic covalent interaction. However, there is the pi-Alkyl interaction present in both complexes, Quercetin (**a**) and Fisetin (**b**), where the pi-Alkyl bond plays a role in strengthening the ligand in the protein through interactions of the pi electrons of an aromatic group on the sigma electrons present in Alkyl groups [93].

For the next complex (Ellagic Acid and Quercetin), the interactions are not very different from the others, except for the number of hydrogen interactions for Quercetin (**d**), in Figure **3**, where the number is greater, a total of four bonds, and the appearance of a pi-sigma interaction (Ala A:168).

Figure **3:** Maps of interactions with amino acid residues in the catalytic site of the PknA protein (PDB ID: 4OW8). (**c**) Ellagic Acid and (**d**) Quercetin.



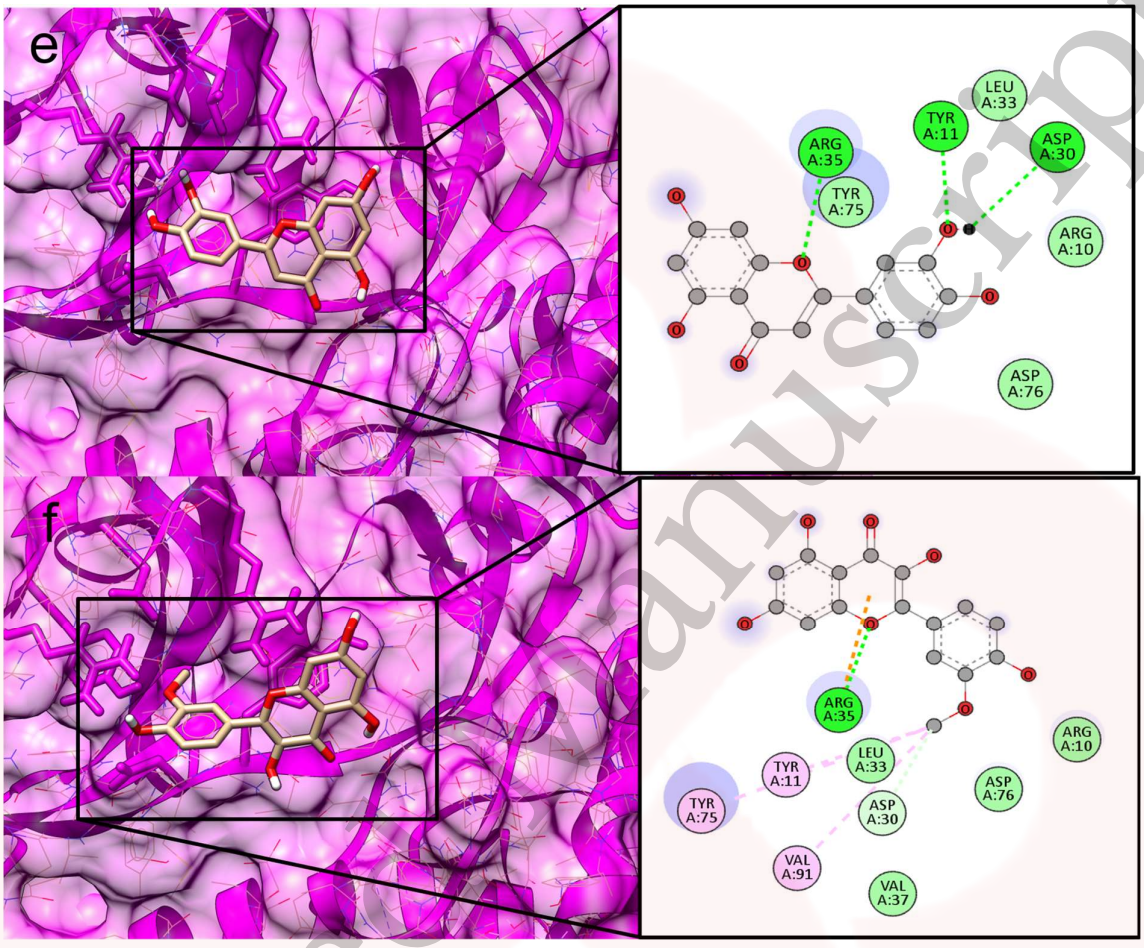
Font: BIOVIA, 2024.

The pi-sigma interaction contributes to the stability and positioning of the ligand, as well as contributing to the binding energy [93]. Ellagic Acid (**c**) does not have any different bonds to the others we have worked on.

For Luteolin (**e**) in Figure **4**, it did not show any more diverse interactions apart from van der Wals interactions and hydrogen bonds; however, Isoharminetin (**f**) showed an Alkyl interaction

in the ether at C1', as well as the pi-Alkyl interactions and carbon hydrogen bond in the same functional group.

Figure **4:** Maps of interactions with the amino acid residues of the catalytic site of the PknB protein (PDB ID: 1MRU). (**e**) Luteolin and (**f**) Isoharminetin.



Font: BIOVIA, 2024.

3.5 Comparison of Binding Energies with Drugs that Fight Diseases

During the docking simulations, it is important to compare the results obtained for the ligands with the binding energies of the drugs used to treat the diseases. The drugs that fight the respective bacterial diseases are Rifampicin, Clofazimine, and Dapsone, whose docking was performed on the same targets using the respective protein coordinates; therefore, their Binding Energy results may be different when using other coordinates and proteins. Their respective docking results, together with the results of the best candidates, are denoted in Table **4**.

Table **4:** Comparison of the binding energies of Aroeira honey x antibiotics used in the treatment of target diseases.

TARGETS	**LIGANDS**						
	Quercetin	Luteolin	Ellagic Acid	Isoharminetin	Dapsone	Clofazimine	Rifampicin
ML2640c	-8.8	-8.6	-8.1	-8.1	-7.2	-8.4	-6.0
PknA	-8.0	-7.7	-8.3	-7.6	-6.3	-9.4	-7.4

PknB	-7.0	-8.1	-8.1	-8.1	-5.8	-8.2	-7.0

Font: AutoDock Vina, 2024.

The highest-scoring ligands from the Aroeira obtained excellent binding energies in relation to the drugs used in the treatments. This suggests that the ligands could be selected for in vitro and in vivo tests in the future, promoting a great future for the ligands in phytopharmaceutical research and eventually more in-depth and accurate tests of the compounds from the Aroeira-do-Sertão.

About the interactions with the amino acid residues of the control drugs, they were not very different from those commented on the ligands of Aroeira, however, some structures showed some interactions in chlorine and sulfur atoms, as well as many hydrogen interactions can be seen in Supplementary figures **1S**, **2S** and **3S**, in which all interactions are denoted.

4 Conclusions

It was observed that the compounds in Aroeira-do-Sertão honey (Quercetin, Luteolin, Ellagic Acid and Isoharminetin) obtained excellent binding energies, as well as very good values for the ADME-Tox parameters used in the work. The aforementioned research on honey highlights the pharmacokinetic potential and the ability of these compounds to interact against important biological targets of leprosy and tuberculosis, as possible candidates for the development of phytopharmaceuticals. However, it is important to emphasize that this is an initial study and requires further scientific investigation with experimental tests, in vitro and in vivo, in later stages to prove their biological activities.

Author's Contribution

E.R.A.S. and R.M.R. suggested the idea of doing a computational investigation. V.M.M.S. and R.F.S.S. performed the computational work. V.M.M.S., G.E.N.C. and E.R.A.S analyzed and interpreted the data. V.M.M.S. wrote the manuscript. E.R.A.S. guided the entire development of the study. All authors reviewed and approved the manuscript.

Availability of Data and Materials

Data is contained within the article.

Consent for Publication

Not applicable.

Conflicts of Interest

The authors declare no conflicts of interest during the writing and research.

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Supportive/Supplementary Material

Download the Supplementary data to this article.

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