IN VITRO AND *IN SILICO* ANALYSIS OF AMENTOFLAVONE AGAINST *Candida albicans* WITH A COMPETITIVE ACTION IN RELATION TO AMPHOTERICIN B

ABSTRACT

Substances isolated from plants with antifungal capacity have received considerable attention from the pharmaceutical sector, due to the phenomenon of resistance and toxicity presented by these microorganisms.
Objectives: Carry out the isolation, identification and characterization of the compound amentoflavone (AMT), as well as evaluate its antifungal activity and modulatory effect against strains of *Candida albicans*, also including the computational study of the mechanism of action of the compound AMT against the Als3 andSap5 enzymes of *C. albicans*.
Methodology: For the isolation of the AMT compound, Thin Layer Chromatography (TLC) was used and its characterization through High Performance Liquid Chromatography (HPLC). Antifungal activity was analyzed using the broth microdilution method. The modulatory activity assays were carried out using the checkerboard technique using AMT and amphotericin B a standard. For the molecular docking study, computer simulations of the interaction between the Als3 and Sap5 enzymes and AMT were carried out using the Vina AutoDock Code
Results: The AMT compound showed an inhibitory effect against all strains of *C. albicans*. In terms of modulatory activity, an indifferent and antagonistic effect was observed for all *C. albicans* strains tested. Molecular docking with AMT showed higher affinity energy for the Als3 and Sap5 enzymes than Amphotericin B. The results obtained in this study suggest that amentoflavone has antifungal effects, as well as high affinity for the Als3 and Sap5 enzymes of *C. albicans*.
Conclusion: These results provide evidence that the AMT compound could be a potential source of a new biotechnological product, acting as a natural antifungal agent.

Keywords: *Amentoflavone;antifungal; modulating effect and natural products.*

1. INTRODUCTION

Fungi are ubiquitous organisms in nature, with most species being harmless to humans. However, *Candida* species are commensal and opportunistic fungi, which under favorable conditions can become pathogenic, developing clinical manifestations known as candidiasis.[1,2].*C. albicans* is one of the most virulent pathogens and one of the main causes of hospital infections that can cause infections ranging from superficial to fatal injuries. Becoming a serious threat to health around the world[3,4,5].

The pathogenicity of *C. albicans* is attributed to many virulence determinants, among which are host tissue adhesion, response to environmental stresses, production of hydrolytic enzymes, transition from yeast to hyphae and production of biofilms. Among the proteins that directly contribute to the pathogenicity of *C. albicans* are the cell surface glycoproteins of the Als family. Among which ALS3 adhesin is commonly associated with fungal adhesion to host cells, as well as more complex interactions, such as invasion and biofilm formation [6,7].

The secreted aspartic proteinases (Saps) are considered putative virulence factors important for the pathogenicity of *C. albicans*[8,9].*C. albicans* has a large gene family encoding Saps, [10]and it is likely that the individual Sap isoenzymes evolved for optimal adaptation to specific functions or host niches[11]. Expression of SAP5 during infection might be activated by signals that also induce hyphal growth, possibly involving the same signal transduction pathways, but might be independent of the hyphal morphology itself[11].

The continuous increase in fungal infections, together with the phenomenon of resistance, has led to the search for new bioactive substances, so that attempts to potentiate the effect of conventional antifungals through interactions between natural products and commercial drugs have been tested against isolates clinical trials and multidrug-resistant *Candida* species. In addition, the development of therapeutic strategies that promote greater selectivity against fungal pathogens, in order to offer better treatment options to patients, are of great relevance for the development of potential candidate molecules for new drugs through molecular docking[12,13]

Naturally occurring structures have been used as targets in the development of new drugs. In this context, we can highlight the plants of the Ochnaceae family, such as *Ourateafieldingiana,* popularly known as batiputá, which is rich in biflavonoids, such as amentoflavone (AMT). This compound presents several promising pharmacological properties, such as antifungal, antioxidant, antitrypanosomal, antiviral, among others. Due to these properties, amentoflavone is a compound that can be exploited for the treatment of several diseases, including those of fungal origin[12,14].

Amentoflavone exhibited potent antifungal activity against several pathogenic fungal strains but had a very low hemolytic effect on human erythrocytes.In particular, amentoflavone induced the accumulation of intracellular trehalose on *C. albicans* as a stress response to the drug and disrupted the dimorphic transition that forms pseudo-hyphae during pathogenesis[15].

Molecular docking is a computational method based on *in silico* structures with great efficiency in studies of molecular recognition processes of possible therapeutic agents. This technique simulates orientation, conformation and interaction modes, making it possible to estimate the interaction energies between two molecules in three-dimensional space, such as protein-protein and protein-ligand, using a potential energy function and a search algorithm as a basis. Furthermore, this method analyzes the affinity of a molecule for a receptor-target binding site, in which the active conformations of molecules and enzymes present geometric and chemical complementarity, essential for the success of therapeutic treatments[12,16].

Given the emergency situation in the development of new therapeutic strategies for the treatment of candidiasis, the present study aimed to evaluate the *in vitro* antifungal activity of the compound amentoflavone (AMT) on *C. albicans* and to evaluate its modulatory potential with the antifungal amphotericin B (AmB), as well as molecular docking studies to analyze the potential use of this biflavonoid as an antifungal pharmacological resource.

2. MATERIAL AND METHODS

2.1 Plant material

The leaves of *O.fieldingiana* were collected in the municipality of Trairi, Ceará, Brazil, in March 2019, at the geographic location (latitude 3° 13'01, 90"S, longitude 39° 23'20, 10" W). A voucher sample (62392) was deposited at the Herbarium Prisco Bezerra of the Federal University of Ceará (UFC) and authenticated by the botanist Luiz Wilson Lima-Verde.

2.1.1 Amentoflavone (AMT) extraction

To prepare the extract, 1 kg of *O. fieldingiana* leaves were dried, ground and macerated in a closed glass bottle at room temperature for seven days with 70% ethanol. The extract was then filtered into a round-bottomed flask and concentrated on a rotary vacuum evaporator at 50°C. After this process, a green material was obtained together with an aqueous solution. The green waxy material was separated from the aqueous solution by filtration in a Buchner funnel coupled to a kitazate and a vacuum pump, and the brownish solution, after lyophilization, resulted in the defatted ethanolic extract of the leaves (EEF) of *O. fieldingiana*, with 16.5% yield[17].

For the isolation of amentoflavone, (AMT), a G-60 silica column was used, which was eluted with ethyl acetate and chloroform solvents in mixtures of increasing polarities. Purification of

the AMT compound was carried out on a sephadex column (LH-20), using the solvent methanol for elution. AMT was identified using Thin Layer Chromatography (TLC), comparing it with an authentic sample previously isolated in our laboratory[18].

2.1.1.1 High performance liquid chromatography (HPLC)

To confirm the purification of the AMT compound, the fraction containing amt was subjected to high performance liquid chromatography (HPLC-DAD). To identify the phenolic compounds, a methanolic solution of the fraction with a concentration of 20 µL mL^{-1} was injected into the equipment. Standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The solvents used for the extraction were of analytical grade (Vetec®), in the analyzes the solvents used were of HPLC grade (J.T. BAKER®). Chromatographic analyzes were performed on a Shim-pack reversed phase column (CLC) ODS GOLD (4.6x250mm, 5µm). Mobile phases C and D were acetonitrile and Milli-Q water acidified to pH 2.8 with phosphoric acid, correspondingly, solvent gradient was used as follows: 0-15 min, an isocratic elution with C:D (20:80 v/v); 17-25 min, linear variation up to C:D (40:60 v/v); 25-40 min, an isocratic elution with C:D (20:80 v/v). The flow rate was 1.0 $mL.min^{-1}$, with an injection volume of 20 μL and a wavelength of 350 nm. The peaks relating to the constituents present in the HPLC chromatogram were confirmed by comparing their retention time with that of the reference standard and by DAD spectra (200 to 400 nm).

2.2 *In vitro* antifungal assay (inoculum preparation for antifungal susceptibility testing)

Antifungal activity was determined by the broth microdilution method, according to the M27-A3 guidelines for yeast, according to the Clinical and Laboratory Standards Institute protocol CLSI M27 –A3, 2008[19]with modifications for natural products. *C. albicans* strains were obtained from the fungal collection of theVale do Acaraú State University. In this study, a total of 05 strains of *C. albicans* were used, 05 strains of clinical isolates of *C. albicans* were used: LABMIC 0101 (blood culture), LABMIC 0102 (blood culture), LABMIC 0104 (tracheal aspirate), LABMIC 0105 (blood culture) and *C. albicans* ATCC 90028 used as the standard strain for the analysis. Microorganisms were grown overnight on potato dextrose agar (Difco, Detroit, MI, USA) and incubated at 37 °C. From this culture, saline-free suspensions were prepared according to the McFarland 0.5 scale. Suspensions were diluted 1:2000 in RPMI 1640 medium (with l-glutamine without sodium bicarbonate buffered to pH 7.0 with 0.165 M MOPS), to obtain an inoculum of $2.5^{-5}x10^{3}$ CFU mL^{-1}.

2.2.1 Broth microdilution method

The minimum inhibitory concentration (MIC) was determined according to the CLSI guideline document. [19,20]amentoflavone (10 mg L^{-1}) was diluted in Dimethylsuffoxide (DMSO) 5%. Amphotericin B (AmB) was prepared in DMSO and placed in concentrations ranging from 16 µg/mL to 0.125 µgmL. For antimicrobial activity, amentoflavone was tested at concentrations ranging from 0.002 to 2.5 mg L^{-1}. The microdilution test was performed in 96-well microdilution plates and incubated at 37 °C, and the antifungal effect was visually analyzed after 24 h.

2.2.2 Synergism by checkboard

The synergistic activity between amphotericin B (AmB) and amentoflavone was determined by the checkboard technique. Initially 50 µL of RPMI-1640 was added to all wells of the 96-well microdilution plate. Then, 50 µL of each dilution of amentoflavone were added in vertical orientation, with concentrations ranging from 5 mg L^{-1} to 0.03 mg L^{-1}. In horizontal orientation, 50 µL of AmB (standard antifungal) were placed at concentrations ranging from 16 µg/mL to 0.125 µg ml, then 100 µL of the *C. albicans* suspension (2.5^{-5} x 10^3 CFU mL -1) were added to all wells and incubated at 37 ºC for 24 h. Assays were performed in triplicate. To interpret the results, the fractional inhibitory concentration index (FICI) was calculated, in which FICI ≤ 0.5 will have a synergistic effect; FICI = 0.5 to 1.0 will be an additive effect; FICI > 1.0 < 4.0 indifferent effects; and FICI > 4.0 antagonistic effects[21,22].

2.3 Molecular Docking (Preparation and optimization of the Amentoflavone (AMT) compound

The two-dimensional AMT and AmB coordinates were obtained from the PUBCHEM repository (https://pubchem.ncbi.nlm.nih.gov/)[23].The structures was rendered using MarvinSketch software (https://chemaxon.com/products/ marvin) and then subjected to structural optimization using the classical force field formalism MMFF94 (Merck Molecular Force Field 94)[24].The optimization calculations were performed using the Avogadro® software, [25]configured to perform force field simulations MMFF94, using the Steepest Descent algorithm, [26] 500 numerical steps and a convergence parameter 10e -7[27].

2.3.1Obtaining the 3D structures of the target proteins

To investigate the antifungal effect of AMT present in the ethanol extract of *O. fieldingiana* against *C. albicans*, the structures of the enzymes Als3 and Sap5 from *C. albicans*were obtained from the Protein Data Bank database (https://www.rcsb.org/), PDB ID: 4LEB and 2QZX respectively [28,29]. In the receptors preparation were added the polar hydrogens and the calculated Gasteiger charges using the software Autodocktools ™ [30,31].

2.3.2 Molecular docking procedures

After the AMT, amphotericin B and enzymes preparation phase, the compound were then subjected to molecular docking. The AutoDock Vina software (version 1.1.2) was used to perform the docking simulations, using 3-way multithreading, exhaustiveness 64 and Lamarkian Genetic Algorithm [32]. Centered on the entire protein, the grid box was defined parameters of 102Åx126Åx92Å and dimensions (x, y, z) = (-5.806, 2.952, -13.754) for the Als3 receptor and parameters of 80Åx82Åx124Å and dimensions (x, y, z) = (20.664, 21.527, 45.515) for the Sap5 receptor. As a standard procedure, 50 independent simulations were performed, obtaining 20 poses each[33]. Redocking procedures were performed for methodological and statistical validation of the simulations. Results analysis was performed using Discovery studio visualizer ™ viewer, PyMOLand UCSF Chimera softwares[34,35].

3. RESULTS AND DISCUSSION

3.1Antifungal activity

The antifungal activity of the AMT (Fig. 1) compound against several strains of *C. albicans* is represented in (Table 1). In the broth microdilution assay against yeast, the compound AMT inhibited the growth of all *C. albicans* strains tested, with values of MIC that ranged from 150 to 1250 µgmL.

Fig.1.A

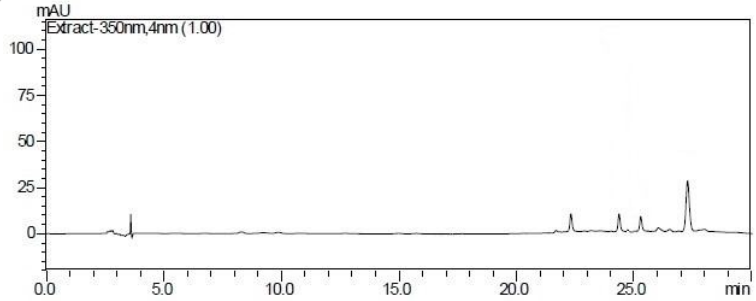

Fig.1.B

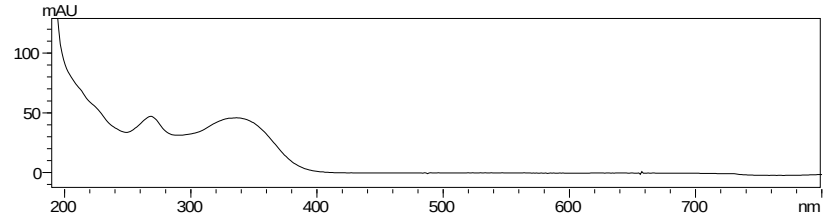

Fig1. **Confirmation of amentoflavone at the retention time of 27.33 obtained on the High Performance Liquid Chromatograph (Fig. 1A) and its ultraviolet (UV) spectrum (Fig. 1B)**

Table 1. Minimum inhibitory concentration of amentoflavone and Amphotericin B

Strains	MIC Amentoflavone (µg/mL)	Amphotericin B (µg/mL)
C. albicans ATCC 90028	150	1.0
C. albicans LABIMIC 0101	620	1.0
C. albicans LABIMIC 0102	1250	1.0
C. albicans LABIMIC 0104	1250	1.0
C. albicans LABIMIC 0105	1250	1.0

**LABMIC: Microbiology Laboratory.*
** MIC Minimum Inhibitory Concentration*

Natural products derived from plants are powerful sources in traditional medicine, due to their low cost, high availability and fewer side effects. In fact, numerous plant-derived bioactive compounds exhibit a wide variety of pharmacological effects, such as amentoflavone. Amentoflavone is found in several plants, and although there are studies in the literature about its biological and pharmacological activities, there are few reports about its antifungal effect against human pathogens, including *Candida* species [15,36].*C. albicans* is the most common human fungal pathogen in clinical cases of invasive fungal infection, and is also associated with oral candidiasis. In the literature, a variety of flavonoids are described as inhibitors of *C. albicans*, including quercetin, kaempferol, rutin and catechin, this being the first report of the antifungal activity of the biflavonoid amentoflavone against *C. albicans*. The presence of these compounds may contribute to the antifungal activity, since these phenolic substances may act in the rupture of the cell membrane, inhibition of cell division, the development of hyphae through targets in specific genes, as well as interference in metabolic pathways and/or induce apoptosis by disrupting redox homeostasis[37,38].

3.1.1 Modulatory Activity

To determine the modulatory activity, 05 strains of *C. albicans* were used. The results are shown in (Table 2). The combination of the AMT compound with AmB did not show a significant reduction in the MIC values of the AMT against *C. albicans* strains. The MIC values of the compound AMT with AmB were the same found for the AMT tested alone against the strains (LABIMIC 0102, LABIMIC 0104 and LABIMIC 0105). While the compound AMT alone showed a better MIC against *C. albicans* strains (LABIMIC 0101 and ATCC 90028), when tested in combination with AmB. From these values, the fractional inhibitory concentration index (FICI) was calculated, which showed an indifferent and antagonistic effect on the modulatory activity for all tested *C. albicans* strains. The MIC values for AmB associated with the compound AMT, despite demonstrating a reduction in the MIC value for amphotericin B, showed antagonistic and indifferent effects against the strains of *C. albicans*. Therefore, suggesting that there was an influence of AMT on the action of AmB, demonstrating that the antifungal effect of both is dependent. Thus, they compete for the same protein virulence targets in the *C. albicans* structure.

Table 2. MIC in the presence and absence of the amentoflavone compound

Strains	Amenthoflavone		Amphotericin B		
	MIC isolated	MIC combined	MIC isolated	MIC combined	FICI
C. albicans ATCC 90028	0.15	1.25	1.0	0.5	8.8
C. albicans LABIMIC 0101	0.62	1.25	1.0	0.5	2.5
C. albicans LABIMIC 0102	1.25	1.25	1.0	0.5	1.5
C. albicans LABIMIC 0104	1.25	1.25	1.0	0.5	1.5
C. albicans LABIMIC 0105	1.25	1.25	1.0	0.5	1.5

**FICI: Fractional Inhibitory Concentration Index; *LABIMIC: Microbiology Laboratory;*
** MIC Minimum Inhibitory Concentration*

Previous studies have shown that the MIC of amphotericin B was reduced when used in combination with quercetin or rutin for the ATCC strain of *Crytococcus neoformans* and reduced when combined with rutin for a clinical isolate of *C. neoformans.* Amphotericin B is an antifungal from the polyene class that selectively binds to ergosterol, which is an

analogue of cholesterol present in mammalian cell membranes, directly interrupting the integrity of the fungal membrane. Thus, its use has been limited in many patients because it almost always results in some degree of renal failure, which varies in severity depending on the total dose [39,40].

Thus, a more promising treatment would be the association of amphotericin B with compounds that enhance its action, so that smaller doses of the drug can be used, or even with compounds that protect target cells or tissues from toxicity mediated by this antifungal. This effect was also observed when amphotericin B was combined with the natural compounds berberine, allicin, epigallocatechin gallate and with essential oils of *Melaleuca alternifolia*, *Origanum vulgare*, *Pelargonium graveolens*, *Coriandrum sativum*, *Thymus maroccanus*, *Thymus broussonetii* and *Lippia alba* against *Candidaspecies* [41-47].The mechanism of action of the synergistic effects with amphotericin and most natural compounds is not well elucidated. However, several studies suggest that subinhibitory concentrations of amphotericin B facilitate the absorption of natural compounds, resulting in increased death of fungal cells [40].

3.2 Molecular Docking

In the*in silico*test, after the molecular docking simulations between the AMT compound and the Als3 (Figure 2) and Sap5 (Figure 3) enzymes from *C. albicans*, it was possible to notice that the AMT presented a RMSD (Root Mean Square Deviation) values within the ideal parameter, less than 2 Å (Table 3)[48]. The affinity energy to evaluate the formation of the complex between AMT, Als3 and Sap5 should present more negative values than -6.0 kcal/mol,[49]which was observed since the AMT compound showed high affinity for the Als3 and Sap5 receptors, -10.5 kcal/mol and -11.3 kcal/mol respectively, evidencing a more favorable affinity energy than the amphotericin B (Table 3).Amphotericin B is an antifungal from the polyene class that selectively binds to ergosterol, which is a cholesterol analogue present in mammalian cell membranes, directly interrupting the integrity of the fungal membrane. Thus, its use has been limited in many patients because it almost always results in some degree of renal failure, which varies in severity depending on the total dose[39,40].The formed complexes presented RMSD in the order of 1.026 Å to 1.707 Å, affinity energy in the order of -11.3 to -7.6 kcal/mol (Table 3). Analyzing the interaction patterns of the AMT compound and AmB against *C. albicans,* it was possible to identify interactions hydrophobic, π-Stacking and hydrogen bonds with distances between 1.85 and 4.45 Å (Table 3).

Table 3. Affinity energy, RMSD values and interaction types of the amentoflavone complex e amphotericin B (control) formed after docking simulations against Als3 and Sap5 *C. albicans*

Als3/Ligand	**Affinity energy (kcal/mol)**	**RMSD (Å)**	**Residue**	**Interaction**	**Distance (Å)**
Amentoflavone	-10.5	1.707	Tyr 21A	Hydrophobic	3.19
			Tyr 21A	Hydrophobic	3.60
			Tyr 23A	Hydrophobic	3.67
			Val 161A	Hydrophobic	3.55
			Val 161A	Hydrophobic	3.81
			Arg 171A	Hydrophobic	3.29
			Lys 59A	Hydrogen Bond	2.39
			Val 172A	Hydrogen Bond	2.66
			Asn 225A	Hydrogen Bond	2.10
			Trp 295A	π-Stacking	4.45
Amphotericin B	-7.6	1.429	Phe 87A	Hydrophobic	3.83
			Ser 125A	Hydrogen Bond	3.22
			Ser 211A	Hydrogen Bond	1.85
			Asn 212A	Hydrogen Bond	2.10
			Val 215A	Hydrogen Bond	2.41
			Glu 231A	Hydrogen Bond	3.18
Sap5/Ligand	***Affinity energy (kcal/mol)***	**RMSD (Å)**	**Residue**	**Interaction**	**Distance (Å)**
Amentoflavone	-11.3	1.026	Ala 162A	Hydrophobic	3.61
			Lys 257B	Hydrophobic	3.61
			Lys 257B	Hydrophobic	3.93
			Pro 290B	Hydrophobic	3.35
			Glu 278A	Hydrogen Bond	3.06
			Gln 282A	Hydrogen Bond	2.00
			Tyr 284A	Hydrogen Bond	3.26
			Tyr 284A	Hydrogen Bond	3.57

			Arg 312A	Hydrogen Bond	2.59
Amphotericin B	-8.4	1.172	Glu 10B	Hydrophobic	3.94
			Asn 160B	Hydrogen Bond	2.76
			Ser 161B	Hydrogen Bond	3.30
			Glu 163B	Hydrogen Bond	2.26
			Glu 163B	Hydrogen Bond	2.98
			Ser 165B	Hydrogen Bond	2.55
			Ser 165B	Hydrogen Bond	3.08
			Thr 166B	Hydrogen Bond	2.06
			Thr 166B	Hydrogen Bond	2.22
			Lys 293A	Hydrogen Bond	2.43

The ALS family constitutes a large group of proteins that includes eight genes that encode large cell surface glycoproteins, of these eight proteins, Als3 is especially important for adhesion and is associated with the pathogenesis of *C. albicans*[50].Thus, Als3 adhesin is considered a promising target involved in the design of antifungals through molecular docking and can be used to prospect molecules capable of inhibiting the function of this protein and the adhesion capacity of *C. albicans*, as well as its pathogenicity, thus providing new anti-*Candida* drugs[51].

Among the most studied isoenzymes related to *Candida* virulence they are Secreted aspartic proteinases (Saps) what are a family of 10 related proteases, among them SAP5. These proteases are virulence factors due to their proteolytic activity, as well as their roles in adherence and colonization of host tissues that participate in the infection process by, degrading several host cell proteins, such immunoglobulins, proteins of the complement system and extracellular matrix, contributing to tissue damage and the resulting invasion by the microorganism [52,53].

The redocking of the co-crystallized hepta-Thr inhibitor against Als3 showed an RMSD value of 1.707 Å and an affinity energy of -10.5 kcal/mol. The sNT-Als3 (shorter version of NT-Als3 that excludes the C-terminal AFR) and hepta-Thr binding site is formed by residues from the A1-B1 loop (Ala 19, Asn 22, Gly 27 and Thr 28), E1 β-strand (Ala 116), A2 β-strand (Tyr 166, Thr 168, Ser170 and Val 172), G2 β-strand (Arg 294, Trp 295, Thr 296 and Tyr 298) e β-strand C1* (Thr 61 and invariant Lys 59).Thus, we observed that amentoflavone binds in

the same region as the binding site of the co-crystallized inhibitor, having in common interactions with residues Lys 59, Val 172 and Trp 295, indicating that has a similar action to hepta-Thr. The results of *in vitro* and *in silico* studies coincide in the assumption that the amentoflavone can act as an effective antifungal compound, including an inhibition of the Als3 enzyme responsible for virulence and adhesion in *C. albicans.* Comparing the amphotericin B (control), we observed that amentoflavone does not compete for the control binding site, thus, confirming the results of *in vitro* assays.

The redocking of the inhibitor Pepstatin A (PepA) co-crystallized in the A and B chains of Sap5 showed an RMSD value of 1.026 Å and an affinity energy of -11,3 kcal/mol. The PepA binding site is formed by residues Ile 12, Asp 32, Gly 34, Ser 35, Lys 83, Tyr 84, Gly 85, Asp 86, Ile 123, Gly 220, Thr 221, Thr 222, Ile 223 and Ile 305 [45]. The analysis of interactions showed that amentoflavone does not compete for the PepA and amphotericin B (control) binding site, thus, confirming the results of in vitro assays.

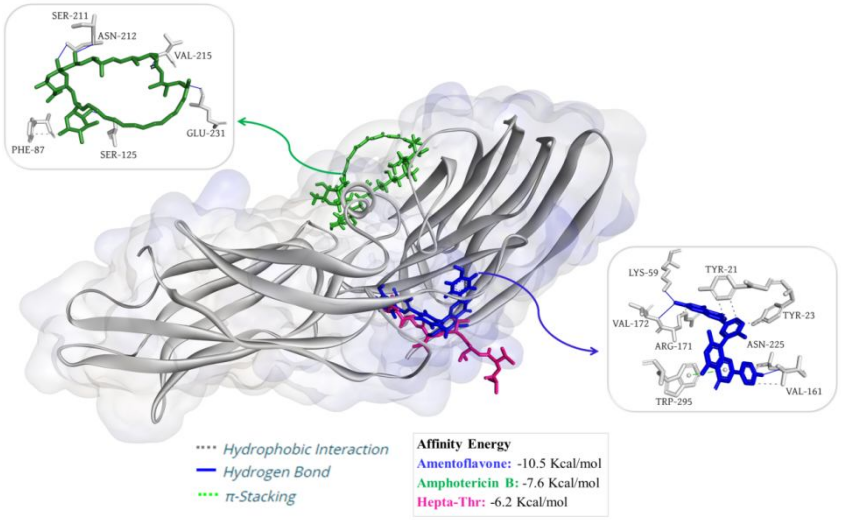

Fig 2. Interaction complex of amentoflavone, antifungal Amphotericin B and Hepta-Thr (co-crystallized inhibitor) with Als3 receptor

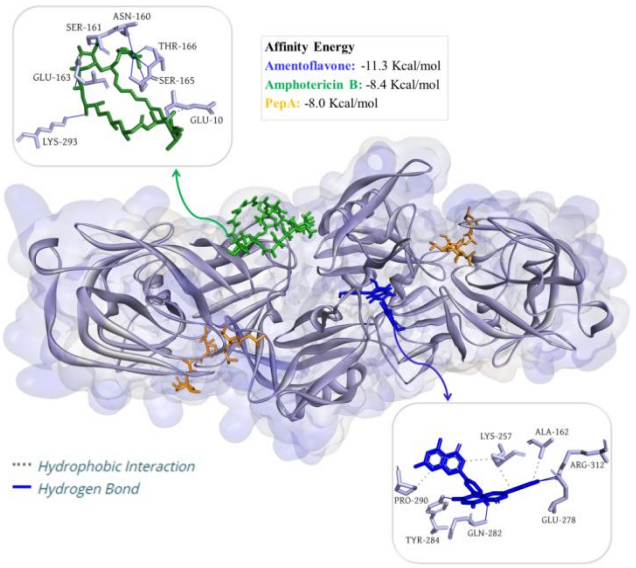

Fig 3. Interaction complex of the amentoflavone, antifungal Amphotericin B and PepA (co-crystallized inhibitor) with Sap5 receptor

4. CONCLUSION

Given the current limitations in antifungal therapy, natural products are attractive prototypes for this purpose, due to their abundance in nature, low cost and broad spectrum of biological activity. Amentoflavone showed antifungal activity against all strains of *C. albicans* with better results than standard amphotericin. The experimental data were corroborated by the molecular docking study, where amentoflavone better affinity energy showed than amphotericin B against *C. albicans* enzymes Als3 and Sap5. Assays of modulatory activity with the combination of AMT and amphotericin B against *C. albicans* showed that they present indifferent actions.

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