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Phenolic profile and antioxidant, anticholinesterase and anti- candida potential evaluation *in vitro* and *in silico* studies of *Tapirira guianensis* Aubl. extracts

Avaliação do perfil fenólico e do potencial antioxidante, anticolinesterase e anti-candida *in vitro* e *in silico* de extratos de *Tapirira guianensis* Aubl.

Evaluación del perfil fenólico y el potencial antioxidante, anticolinesterásico y anti-candida *in vitro* e *in silico* de extractos de *Tapirira guianensis* Aubl.

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Abstract

Polar extracts of *Tapirira guianensis* Aubl. were prepared for evaluation of biological activities. Identification and quantification of phenolic compounds, antioxidant activity, inhibition of acetylcholinesterase enzyme and antifungal potential were performed in all extracts. High contents of total phenols were detected in the ethanolic and aqueous extracts of the leaves (237.10±5.39 mg EAG/g and 346.46±5.67 mg EAG) respectively. The same extracts had a free radical scavenging potential by both DPPH and ABTS+ methods. Ethanolic extract of the leaves with IC_{50}= 15.06±0.02 µg/mL and 16.49±0.01µg/mL) and aqueous extracts (11.54±0.01 µg/mL and 15.14±0.02 µg/mL respectively. In the study by high-performance liquid chromatography the phenolic substances were recorded: gallic acid, rutin, ellagic acid, quercetin and apigenin in the leaves and gallic acid and quercetin in the stem bark. Ethanolic

and aqueous extracts of the leaves and stem bark showed high potency of inhibiting the enzyme acetylcholinesterase, with IC_{50} values = 13.94±0.02 µg/mL, 13.69±0.08 µg/mL and 14.79±0.20 µg/mL respectively. The aqueous extract of the leaves showed anti-Candida action against all four strains of the microorganism. The in silico studies evidenced that all the ligands analyzed presented interaction with the SAP5 protein highlighting the ellagic acid, the rutin and the quercetin. With these results, the activities previously reported for the species *T. guianensis* are confirmed, highlighting the polar extracts, mainly aqueous as a source of antioxidant, acetylcholinesterase inhibitor and antifungal substances.
Keywords: *Tapirira guianensis*; Antioxidant; Acetylcholinesterase; Anti-Candida.

Resumo
Extratos polares de *Tapirira guianensis* Aubl. foram preparados para avaliação de atividades biológicas. Identificação e quantificação de compostos fenólicos, atividade antioxidante, inibição da enzima acetilcolinesterase e potencial antifúngico foram realizados em todos os extratos. Foram detectados teores elevados de fenóis totais nos extratos etanolicos e aquosos das folhas (237,10±5,39 mg EAG/g e 346,46±5,67 mg EAG) respectivamente. Os mesmos extratos apresentavam um potencial de extração de radicais livres tanto pelo método DPPH como pelo método ABTS+. Extrato etanólico das folhas com CI_{50}= 15,06±0,02 µg/mL e 16,49±0,01µg/mL) e extratos aquosos (11,54±0,01 µg/mL e 15,14±0,02 µg/mL respectivamente. No estudo por cromatografia líquida de alta eficiência foram registradas as substâncias fenólicas: ácido gálico, rutina, ácido elágico, quercetina e apigenina nas folhas e ácido gálico e quercetina na casca do caule. Extratos etanólicos e aquosos das folhas e casca do caule mostraram uma elevada potência de inibição da enzima acetilcolinesterase, com valores de CI_{50} = 13,94±0,02 µg/mL, 13,69±0,08 µg/mL e 14,79±0,20 µg/mL respectivamente. O extrato aquoso das folhas mostrou ação anti-Candida contra as quatro cepas do microrganismo. Os estudos in silico evidenciaram que todos os ligantes analisados apresentaram interação com a proteína SAP5 destacando o ácido elágico, a rutina e a quercetina. Com estes resultados, confirmam-se as atividades anteriormente relatadas para a espécie *T. guianensis*, destacando-se os extratos polares, principalmente aquosos como fonte de antioxidante, inibidor da acetilcolinesterase e substâncias antifúngicas.
Palavras-chave: *Tapirira guianensis*; Antioxidante; Acetilcolinesterase; Anti-Candida.

Resumen
Se prepararon extractos polares de *Tapirira guianensis* Aubl. para evaluar las actividades biológicas. En todos los extractos se realizó la identificación y cuantificación de los compuestos fenólicos, la actividad antioxidante, la inhibición de la enzima acetilcolinesterasa y el potencial antifúngico. Se detectaron altos contenidos de fenoles totales en los extractos etanólico y acuoso de las hojas (237,10±5,39 mg EAG/g y 346,46±5,67 mg EAG) respectivamente. Los mismos extractos tenían un potencial de barrido de radicales libres por los métodos DPPH y ABTS+. Extracto etanólico de las hojas con CI_{50}= 15,06±0,02 µg/mL y 16,49±0,01µg/mL) y extractos acuosos (11,54±0,01 µg/mL y 15,14±0,02 µg/mL respectivamente. En el estudio por cromatografía líquida de alto rendimiento se registraron las sustancias fenólicas: ácido gálico, rutina, ácido elágico, quercetina y apigenina en las hojas y ácido gálico y quercetina en la corteza del tallo. Los extractos etanólicos y acuosos de las hojas y de la corteza del tallo mostraron una alta potencia de inhibición de la enzima acetilcolinesterasa con valores CI_{50} = 13,94±0,02 µg/mL, 13,69±0,08 µg/mL y 14,79±0,20 µg/mL respectivamente. El extracto acuoso de las hojas mostró una acción anti-Candida contra las cuatro cepas del microorganismo. Los estudios in silico evidenciaron que todos los ligandos analizados presentaron interacción con la proteína SAP5 destacando el ácido elágico, la rutina y la quercetina. Con estos resultados, se confirman las actividades previamente reportadas para la especie T. guianensis, destacando los extractos polares, principalmente acuosos como fuente de sustancias antioxidantes, inhibidoras de la acetilcolinesterasa y antifúngicas.
Palabras clave: *Tapirira guianensis*; Antioxidante; Acetilcolinesterasa; Anti-Candida.

1. Introduction

The species *Tapirira guianensis* Aubl is one of the species of the genus *Tapirira* most distributed in all Brazilian biomes and is usually presented as "pau-pombo" or "pigeon breast". T. guianensis is a tree that can reach 8-14 meters in height and is established in moist soils in Brazil, mainly in areas of the Atlantic Forest. Its leaves are used in folk medicine to treat leprosy, diarrhea, and syphilis (David et al., 1998). The leaves of T. guianensis present antioxidant and vasodilator activity (Rodrigues et al., 2017), anticholinesterase (Morais et al., 2021), antiproliferative (Silva-Oliveira et al., 2021). Barks exhibit cytotoxic activity against human prostate cancer, antiprotozoal and antibacterial activity (Roumy et al., 2009).

Some therapeutic lines have been applied in individuals affected by Alzheimer's disease. Among them are the use of acetylcholinesterase inhibitors and antioxidant substances. These acetylcholinesterase inhibitors (ACEIs) act by inhibiting the

enzyme acetylcholinesterase, hindering the hydrolysis of acetylcholine, favoring better levels of the neurotransmitter in the cholinergic complex, enabling more effective and prolonged nerve synapses (Araújo; et al., 2016).

In several pre-clinical and clinical studies it is already evident that oxidative stress leads to several neurodegenerative diseases. Oxidative stress consists of an imbalance in the production of reactive oxygen species and antioxidant agents, generating an accumulation of free radicals in the body. Excessive free radicals can lead to cell membrane damage through lipid peroxidation, structural and functional alteration of proteins, DNA damage, and premature cellular aging, causing neurodegenerative problems (Kim et al., 2015). Strategies using antioxidants can help slow or minimize oxidative damage. Polyphenolic antioxidants are natural substances widely used to protect cellular components. Among these polyphenolic substances, flavonoids promote neuroprotective properties in preclinical models of Alzheimer's disease (AD) and Parkinson's disease (PD).

The prevalence of multidrug-resistant microbial infections has also become a major public health problem worldwide. Pathogen resistance to drugs used by the hospital industry is seen as a major threat. In this regard, research into natural antimicrobial agents is expanding worldwide. Antibiotic resistant bacterial strains are responsible for 60% of nosocomial infections (Trubiano & Padiglione, 2015) and Candida yeasts are frequently isolated from intensive care unit (ICU) patients (Schelenz, 2008).

From the above, the present study aims to propose that the species *Tapirira guianensis* Aubl. be registered as a natural source of antioxidant, anti-cholinesterase and anti-candida substances, which can be used in the management of Alzheimer's disease and Candida infections.

2. Methodology

Plant extracts

The collection of plant material was carried out at Parque Estadual do Cocó in Fortaleza-CE in 2021 (Lat.: -3.74462, Long.: -38.48782). The leaves and stem bark of the species *Tapirira guianensis* Aubl. were packed in plastic bags and sent to the Natural Products Chemistry Laboratory of the State University of Ceará (UECE). An exsicata of the plant material was deposited in the Prisco Bezerra Herbarium of the Federal University of Ceará (UFC) identified by the botanist Luiz Wilson Lima-Verde under code EAC 64238.

The license to collect the plant material at Parque do Cocó was granted by the Secretary of Environment of the Government of the State of Ceará. For the processing of the alcoholic extracts, 500 grams of dried sample was used, where it was added to glass containers containing 2L of ethyl alcohol (96%) within 7 days. For the aqueous extracts, the plant material was processed using a reflux system containing 3L of distilled water for 6 hours. Obtaining the resulting solutions, the material was filtered and concentrated in a rotary evaporator under reduced pressure to eliminate excess solvent. To finalize the material, the solutions were concentrated in a water bath (60°C). The aqueous solutions were lyophilized.

Total phenol content

The determination of the total phenol content was carried out using the Folin-Ciocalteu method described by Sousa et al. (2007). For each extract, 7.5 mg was dissolved in 10 mL of P.A. methanol (99.8%) using an ultrasonic bath, then transferred quantitatively to a 25 mL volumetric flask and the final volume was made up with methanol. A 100 µL aliquot of this solution was transferred to a 10 mL volumetric flask with 500 µL of the Folin-Ciocalteu reagent and stirred for 30 seconds, 6 mL of distilled water and 2 mL of Na_2CO_3 (15%) were added, stirring the mixture for more 60 seconds and the final volume was filled with distilled H_2O, the solution was kept at rest for 2 hours in a dark place. The white reagent was conducted under the same conditions. All determinations were made in triplicate. The same procedure was used to prepare the calibration standard

curve (y = 0,127x + 0,011, R^2 = 0,995) obtained with 0 a 4 µg mL^{-1} gallic acid solutions. The absorbances from the several concentrations were obtained in the UV-Vis (Genesys 10S UV-Vis Thermo Scientic) at 750 nm. The results were determined by interpolation of the data with the gallic acid calibration standard equation and expressed in terms of mg GAE/g sample extract.

Total Flavonoid Content

It was evaluated by the method of (Funari & Ferro, 2006) for this test a solution was prepared with 20 mg of extract in 10.0 mL of ethanol using a volumetric flask. After that, a 2.0 mL aliquot of this solution (concentration of 2 mg/mL) was mixed with 1.0 mL of a 2.5% aluminum chloride solution in a 25.0 mL volumetric flask, and then the final volume was made up with ethanol. After 30 minutes resting in the dark and at room temperature, the absorbance of the sample was determined at 425 nm in a UV-Vis spectrophotometer. The blank was prepared in a similar way containing only ethanol and 2.5% aluminum chloride. The procedure was carried out in triplicate. Total flavonoid content was determined by interpolation of the absorbance of the samples against a calibration standard curve constructed by measuring absorbances of quercetin (0 to 14 µg/mL) and expressed as mg EQ (quercetin equivalents) per gram of extract. The equation of the quercetin calibration curve constructed was: y= 0.067x - 0.011 with R2=0.999.

High Performance Liquid Chromatography

The analysis by high-performance liquid chromatography (HPLC-DAD) uses a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan) equipped with Shimadzu LC-20AD reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector, and LC Solution 1.22 SP1 software. Chromatographic analyses were performed on a Shim-pack (CLC) ODS GOLD reverse phase column (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 20μL and wavelength of 350 nm. The chromatography peaks were confirmed by comparing their retention time with that of the reference standard and by DAD spectra (200 to 400 nm). Calibration curve for gallic acid: y= 3.10-8x - 0.0061 (r=0.9997), for rutin: y= 4.10-8x + 0.005 (r=0.999), for ellagic acid: y=8. 10-9x - 0.0159 (r=0.999), for quercetin: y= 2.10-8x - 0.0171 (r=0.988), for apigenin: y= 1.10-8x + 0.0001 (r=0.999). The samples were analyzed in triplicate and the average peak areas were measured.

Determination of antioxidant activity by the DPPH Method

The antioxidant activity was measured in 96-well flat-bottom plates using an Elisa BioTek reader, model ELX 800, software “Gen5 V2.04.11”, based on the methodology described by (Becker et al., 2019) with modifications. In 96-well plates, the following solutions were used per well: 180 µL of methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl), 20 µL of the extract sample dissolved in methanol and diluted 10 times to obtain final concentration 0.2 mg mL^{-1}.

Various concentrations of the samples were prepared using the initial solution with a concentration of 2 mg mL^{-1}: 200 µg mL^{-1}, 100 µg mL^{-1}, 50 µg mL^{-1}, 25 µg mL^{-1}, 12.5 µg mL^{-1}, 6.25 µg mL^{-1}, 3.12 µg mL^{-1}, 1.56 µg mL^{-1}, and 0.78 µg mL^{-1}. Absorbances were measured at 490 nm until a total of 60 minutes of incubation. As the negative control, a solution with all reagents except the sample. The absorbances relative to the extract’s colors were extinguished from the analysis. BHT antioxidant was used for comparison.

The results are expressed as percentage of inhibition, calculated by PI% = [(AC-AS)/AC].100, where AC is absorbance of the DPPH* control solution at time 0 and AS is the absorbance of the sample solution containing DPPH* at time 60 min. All samples were analyzed in triplicate.

Assessment of Antioxidant Activity by the ABTS Method

Antioxidant activity was assessed using the ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) method, as described by (Re et al., 1999). The $ABTS^{*+}$ solution (7 mM, 5 mL) was mixed with 88 μL of potassium persulfate (140 mM), agitated, and kept in the dark at room temperature for 16 hours. Then, 1mL of this solution was added to 99 mL of ethanol, and the absorbance was measured at 734 nm. A series of solutions of the plant extracts with decreasing concentrations was prepared, and 3.0 mL of $ABTS^{*+}$ solution was added to 30 μL of these solutions after 6 minutes. The absorbance was then measured at 734 nm. The IC_{50} values were calculated by linear regression. All samples were analyzed in triplicate.

In vitro evaluation of acetylcholinesterase inhibition

The acetylcholinesterase quantitative enzyme inhibition test used the following reagents per well: 25 µL of acetylthiocholine iodide (15 mM), 125 µL of 5.5'-dithiobis-[2-nitrobenzoic] in the 0.1 M Tris/HCl solution NaCl and 0.02 M $MgCl_2$.$6H_2O$, 50 µL of Tris/HCl solution with 0.1% bovine serum albumin, 25 µL of samples/standards, the standards of physostigmine (Eserina) and Galantamina, separately, and 25 µL of AChE (0.22 unit,uL^{-1}). The inhibitory activity of the acetylcholinesterase enzyme (AChE) was measured in 96-well flat-bottom plates using an ELISA BIOTEK reader, model ELX 800, software "Gen5 V2.04.11". based on the methodology described by (Ellman et al., 1961) modified by Trevisan et al. (2003).

To determine whether the positive results presented in the microplate assay were due to enzymatic inhibition or inhibition of the chemical reaction between DTNB and thiocholine, the reaction kinetics of each well were observed, and the color of the samples was extinguished from the analysis by reading the microplate before and after enzymatic addition. The same analyzes were also performed with the positive control (physostigmine), the negative control (all solutions except the sample and/or standard) and the blank (all solutions except the sample and/or standard + methanol). All samples were diluted in methanol, prepared in 2 $mg.mL^{-1}$ solutions and IC_{50} calculated from the following curve of evaluated concentrations: 200 $\mu g.mL^{-1}$, 100 $\mu g.mL^{-1}$, 50 $\mu g.mL^{-1}$, 25 $\mu g.mL^{-1}$, 12,5 $\mu g.mL^{-1}$, 6,25 $\mu g.mL^{-1}$, 3,12 $\mu g.mL^{-1}$, 1,56 $\mu g.mL^{-1}$, e 0,78 $\mu g.mL^{-1}$. The values obtained were also converted from µg/mL to mol/L, with the exception of the value for the extract.

In vitro antifungal activity

Initially, a screening was performed to verify which samples showed action against *Candida albicans* strain 0102 (clinical). The strains used from the University of Vale do Acaraú collection were from isolates from patients at the Santa Casa de Misericórdia de Sobral, Ceará, Brazil. After screening, the samples that showed activity against the microorganism were tested against *Candida albicans* strains: M1(clinic); C3(clinic); 0102(clinic); 0104 (clinic).

Minimum Inhibitory concentration (MIC) and Minimum fungicidal concentration (CFM)

The Minimum Inhibitory Concentration (MIC) for *Candida. albicans* strains were determined by the broth microdilution broth microdilution method according to the methodology described by (Fontenelle et al., 2007), with the standards of the protocol Clinical Laboratory Standards Institute - M38-A (CLSI, 2018). The samples were diluted (10 mg/mL) in 5 µl of 5% dimethyl sulfoxide (DMSO) 5% DMSO, together with 995 µl of tween. Amphotericin B was used as a positive control. For this assay, 96-well microdilution plates were used, where initially 50 µL of RPMI medium was added to all wells

and then 10 mg/mL of the sample was added to all wells of the first well. added to all wells of the first column to then make serial dilutions. Finally, 50 μL of the inoculum was added to the wells of the plate.

The plates were incubated at 37º C and visual reading was performed after 48 hours for the strains of *C. albicans*. All tests were performed in duplicate and the MIC was defined as the lowest concentration concentration of the sample capable of inhibiting 100% of the visible growth of the microorganism. The results were determined by visualization as recommended by CLSI. A minimum fungicidal concentration (MFC) was determined by subculturing 100 μL of solution removed from wells, without turbidity, on Potato Dextrose Agar medium at 28 °C. THE CFM was determined as the lowest concentration that resulted in no growth in the subculture after 48 hours.

Molecular Docking Methodology

Ligand preparation and optimization

The chemical structure of the phytochemicals apigenin (CID5280443), ellagic acid (CID5281855), gallic acid (CID370), quercetin (CID5280343) and rutin (CID5280805) were obtained from the PubChem repository(https://pubchem.ncbi.nlm.nih.gov/),the lower energy confomers were saved using the MarvinSketch code (Csizmadia, 1999) and optimized using the Avogadro code (Hanwell et al, 2012), configured to use steepest descent algorithm with 50-interaction cycles, applying the Merck Molecular Force Field (MMFF94) (Halgren, 1996; de Andrade Neto et al., 2021).

To investigate the mechanism of action of phytochemicals against *Candida albicans* (da Silva et al., 2021), the structure of "Secreted aspartic proteinase (Sap) 5" was selected from the Protein Data Bank (https://www.rcsb.org/), PDB 2QZX, resolution 2.5 Å (Borelli et al., 2008). In the target preparation step, residues were removed, polar hydrogens added and Gasteiger charges calculated (Yan et al., 2014) using the Autodocktools ™ code (Huey, et al., 2014).

Molecular Docking Simulationand Data Output

We performed 50 molecular docking simulations using the proposed methodology (Da Silva et al., 2022), where the generated grid parameters were centered in order to involve the entire enzyme structure using the axes (20.664 x, 21.527 y, 45.515 z), size (80 x, 82 y, 124 z) and exhaustiveness 64 (Marinho et al., 2020) using the AutoDockVina code (Trott & Olson, 2010). To obtain comparative data, molecular docking simulations were performed with Fluconazole.

The program generated 20 poses for each simulation, which were subsequently selected based on the statistical parameter RMSD (Root Mean Square Deviation) with values up to 2.0Å (Yusuf et al., 2008) and affinity energy less than -6.0 kcal/mol (Shityakov & Förster, 2016; Silva, et al., 2021).

Validation of the Docking Method

To validate the docking molecular simulations, the aspartic protease inhibitor Pepstatin A (PepA) co-crystallized with SAP5 was subjected to the redocking technique. The generated complex was compared to the native complex by superimposing structures to confirm the reliability of molecular docking and coding used as a tool to accurately simulate receptor-ligand interactions (Antonopoulou et al., 2022).

Visualization of Binding Modes and SAP5–Ligand Interactions

The results were visualized using UCSF Chimera ™ (Pettersen et al., 2004). The complexes were saved in PDB format using PyMOL (Schrödinger, 2019). The molecular interactions and seniorelated hydrogen bonds were calculated by Discovery studiovisualizer ™ viewer (Boivia, 2016). We evaluated hydrogen bonds (H-bonds) based on their intensity using

the values of the distances between the donor and recipient atoms, where Strong bonds have a distance between 2.5-3.1 Å, Average bonds have distances between 3.1-3.55 Å, and Weak bonds have distances greater than 3.55 Å (Imberty et al., 1991).

3. Results and Discussion

The phenol content in the extracts was high according to literature analyses (Morais et al., 2021). It is known that the presence of high phenol content may be an indication for the anti-diarrheal action of the plant (Rao & Camilleri, 2010). The antioxidant activity is related in the protection of many physiological and neuro-degenerative disorders such as Alzheimer's, heart diseases, premature aging (Duthie, et al., 2000). Acetylcholinesterase (AChE) is the enzyme responsible for hydrolyzing the neurotransmitter acetylcholine (ACh) at cholinergic synapses. At these synapses ACh acts by transmitting the message from one neuron to another. Cholinergic synapses are widely distributed in the central nervous system (CNS) and peripheral nervous system (PNS), and are important for the maintenance of numerous human physiological functions). In neurodegenerative diseases such as Alzheimer's, anticholinesterase drugs are used to decrease the symptoms of patients affected with the disease (Araújo, Santos & Gonsalves, 2016). Therefore, the data obtained demonstrate T. guianensis as a source of active principles with cholinesterase action, which may participate in the production of new anticholinesterase drugs.

Content of phenols, flavonoids and antioxidant potential

The leaves of *T. guianensis* are rich in phenolic compounds emphasizing the aqueous extract (346, 22 ± 5, 67 mg EAG/g). For the ethanolic extract of the leaves, we obtained (237.10±5.39 mg EAG/g) of total phenols. Using the solvent ethanol, our study was able to quantify a higher content of phenols compared to the study of (Morais et al., 2021) who used the same solvent. The contents of polyphenols, flavonoids and flavanols in H2O, methanol and hydromethanolic extracts of T. guianensis were verified by (Patient et al., 2022). When comparing the H2O extract with our study, higher contents of total phenols could be verified, compared to the authors' study. For flavonoids we obtained (15.76±0.15 mg EAQ/g) when compared to (16.7 ± 6.3mg CE/g DM) from the literature. Analyzing the CI_{50} data of both DPPH radical and ABTS+ radical it is possible to see that the leaves hold antioxidant potential (Table 1). The present study found a IC_{50}= 15, 06±0, 02 µg/ml for the ethanolic extract of *T. guianensis* leaves, a value slightly above (IC_{50}=13.20 ± 0.69 µg/ml) found by (Morais et al., 2021).

Table 1. Total phenol content, flavonoids and antioxidant potential of *T. guianensis* Aubl extracts.

Samples	Total Phenols (mg EAG/g)	Total Flavonoids (mg EAQ/g)	IC_{50} DPPH (µg/mL)	IC_{50} $ABTS^{+}$ (µg/mL)	IC_{50} AChE (µg/mL)
EEFO	237,10±5,39	90,95±0,84	15,06±0,02	16,49±0,01	13,94±0,02
EECC	116,36±3,27	6,97±0,12	20,10±0,03	24,68±0,02	14,79±0,20
EAFO	346,46±5,67	11,41±0,31	11,54±0,01	15,14±0,02	13,69±0,08
EACC	163,60±7,53	4,57±0,13	34,96±0,04	33,77±0,01	28,24±0,09
QUERC	-	-	1,05 ± 0,55	0,95 ± 0,06	-
PHYSO	-	-	-	-	1,15±0,05

All tests were performed in triplicate and the results were expressed as mean ± standard deviation. EEFO: ethanolic extract from the leaves; EECC: Stem bark ethanolic extract; EAFO: aqueous leaf extract; EACC: aqueous bark extract; DPPH: 2,2-diphenyl-1-picrylhydrazyl; QUERC: quercetin (control); PHYSO: physostigmine (control); IC_{50}: Concentration required to inhibit fifty percent of the radical. Source: Authors.

It is interesting to note that the samples that obtained higher levels of phenolic compounds promoted better potential for scavenging free radicals (DPPH and $ABTS^{+}$) and inhibiting the enzyme acetylcholinesterase, corroborating the relationship between these compounds and the tested biological activities already mentioned by several other authors.

High Performance Liquid Chromatography

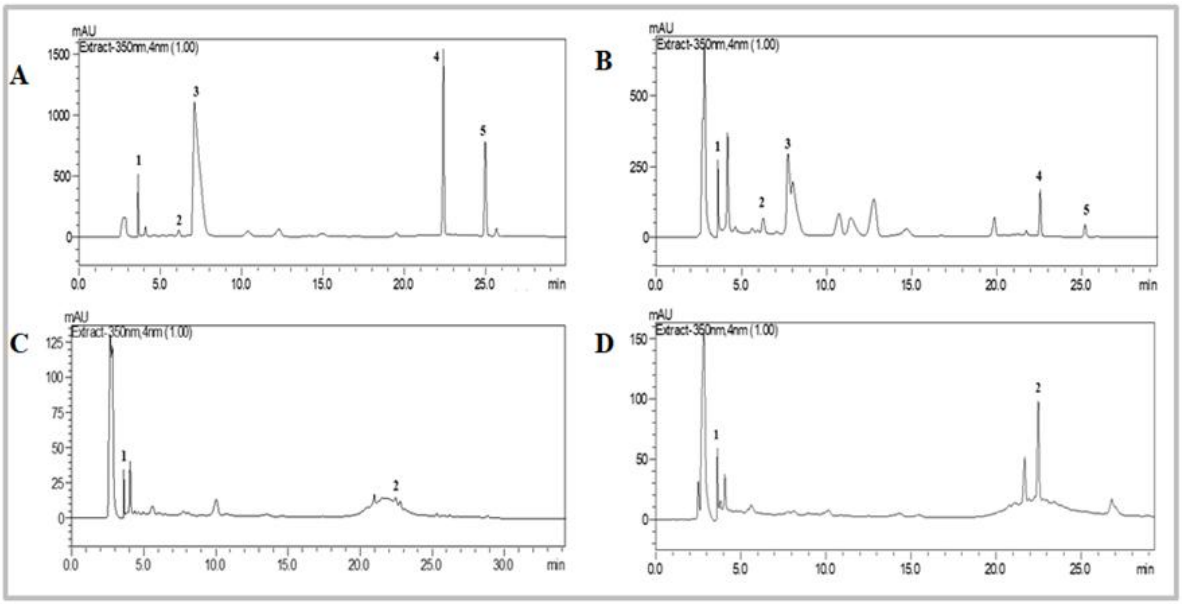
The substances detected in the leaf extracts were similar due to the close polarities of the solvents used in the extraction. Among the substances identified are: gallic acid, ellagic acid and the flavonoids: rutin, quercetin and apigenin. In the barks of the T. guianensis stem, two phenolic compounds were identified: gallic acid and quercetin (Table 2) (Figure 1).

Table 2 Identification and quantification of polyphenols (mg/g Extract) in polar extracts of *T. guianensis* by High Performance Liquid Chromatography.

Compounds	Retetion Time (min)	EEFO	EAFO	EECC	EACC
Gallic Acid	3.57	2,06±0,06	1,57±0,01	0,05±0,00	0,01±0,003
Rutin	6.23	1,59±0,01	2,46±0,02	-	-
Ellagic Acid	7.44	11,24±0,26	1,03±0,18	-	-
Quercetin	22.44	10,29±0,06	0,42±0,04	0,24±0,01	0,32±0,07
Apigenin	24.76	3,05±0,09	0,20±0, 001	-	-

EEFO: Leaf ethanolic extract; EECC: Bark stem ethanolic extract; EAFO: Leaf aqueous extract; EACC: Bark stem aqueous extract. Source: Authors.

Figure 1. Chromatographic profile of ethanolic extract of leaves (A) aqueous extract of leaves (B) ethanolic extract of stem bark(C) and aqueous extract of stem bark(D).



Source: Authors.

As the presence of gallic acid, quercetin, rutin, ellagic acid and apigenin compounds was suggested through high performance liquid chromatography, we can justify the antioxidant potential of the extracts, since several authors have evidenced the antioxidant potential for the substances below, highlighting ellagic acid and quercetin with the highest levels in T. guianensis leaves. Furthermore, the presence of the substances rutin, ellagic acid and apigenin in T. guianensis had not been previously mentioned in the literature.

Gallic acid is classified as a phenolic acid (trihydroxyl - benzoic acid). It exhibits anticancer property as it induces apoptosis of lung cancer cells and inhibits tumor growth (Ohno et al., 1999; Raina et al., 2008). Considered a potent radical ion scavenger and metal chelator (Badhane, et al., 2015), moreover, it has already been reported with activity against three types of *Candida* yeasts (*C. albicans*, *C. glabrata* and *C. tropicalis*), with the *C. albicans* type being the most sensitive with MIC = 12.5 μg mL-1 (Li et al., 2017). The presence of gallic acid compound had already been mentioned in the flowers of T. guianensis (Silva et al., 2020), however, the present study was able to suggest the presence of the compound both in the leaves and in the stem bark of the species, moreover, the antioxidant potential of the extracts can be justified by the presence of the constituent, since there are already several reports on the antioxidant potential of the constituent in the literature.

Rutin (3,3',4',5,7-pentahydroxyflavone-3-ramnoglycoside) is considered a flavonol-type bioflavonoid that exhibits several pharmacological properties. Among them are antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anti-adipogenic, neuroprotective and anticancer (Yang, et al., 2008; Ivanov et al., 2020). Regarding the antifungal potential for rutin, action of the compound was evidenced against four strains of *C. albicans*, obtaining MIC= 37.5 μg mL-1 (Imani et al., 2021). The ethanolic and aqueous extracts of T. guianensis promoted an excellent antioxidant action against the radicals analyzed. Since rutin already presents antioxidant action described in the literature and its presence was detected by high performance liquid chromatography in the leaves, we can justify the antioxidant action of the leaves by its presence.

Ellagic acid is a natural polyphenol that has proven antioxidant, antifibrosis, and anti-inflammatory properties (Gupta et al., 20231; Ren, et al., 2021). Ellagic acid also shows activity against *Candida*. Tested against *C. auris*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. albicans* strains, the compound obtained MICs in the range of 0.12 to 0.50 μg mL-1 (Zhang et al., 2020).

Quercetin (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one) is a flavonol type flavonoid that has been well described by authors who have studied the T. guianensis species. This flavonoid has several pharmacological properties, among them we can mention: anticancer, anti-inflammatory, antidiabetic, antibacterial, antiviral, anti-ulcer and mainly antioxidant (Kwun & Lee, 2020; Sharma et al., 2018; D'Andrea, 2015). It shows potential against C. albicans already reported by (Wang et al., 2016), against 50 strains isolated from gynecological patients in Poland, where the MICs ranged from 2 to 256 μg mL-1. The flavonol quercetin has already been detected in other studies of *T. guianensis*, both in leaves and flowers, as well as its free radical scavenging property. Thus, we can affirm that the antioxidant action of T. guianensis extracts is also related to the presence of quercetin.

Another compound detected by HPLC in the T. guianensis species was apigenin (4',5',7,-trihydroxyflavone), which is classified as a flavonoid of the flavone type. This flavone is already described in the literature with antioxidant, anti-inflammatory, antigenotoxic, antiproliferative, antiangiogenic, cardiovascular, antimutagenic, and anticancer activities (Janeczko et al., 2022; Yan et al., 2017). In addition to the above benefits, it also has chemopreventive potential against various cancers with low intrinsic toxicity (Singh et al., 2022). Antifungal activity already detected for the compound against four *Candida* strains. *C. krusei*, *C. albicans*, *C. glabrata* and *C. tropicalis* with MICs in the range of 100 ± 0.10 μg mL-1 - 150 ± 0.20 μg mL-1 (Smiljkovic et al., 2017). The present study suggests the presence of novel substances for the phenolic profile of the leaves of the species T. guianensis.

Analyzing the structure of flavonoids identified in the leaves of T. guianensis it is possible to justify the excellent antioxidant potential of the leaves. The flavonoids rutin and quercetin present in the structure the ortho-dihydroxy or catechol group in the B ring, conferring high stability to the radical formed after the hydrogen donation from one of the hydroxyls. Both flavonoids also present in their structure the conjugation of the B ring with the 4-oxo group through the 2,3 double bond, promoting the delocalization of the B ring electrons. Another feature is the presence of the 3- OH and 5-OH groups with the 4-oxo group, allowing electron delocalization between these groups in the quercetin structure (Bors et al., 1987).

The in silico antioxidant potential of the flavonoids rutin, quercetin and apigenin were compared with the standard gallic acid. Using the DPPH method, rutin and quercetin are similar to gallic acid. However, through the ABTS*+ method, rutin and quercetin promoted more promising results. Apigenin showed lower results when compared to gallic acid standard (Frota et al., 2022).

Extracts of 42 medicinal plants of Camarrões presented IC_{50}< 50 µg.mL-1 present high antioxidant activity, already for samples that obtained 50< IC_{50} <100µg.mL-1 promoted moderate activity and for samples with IC^{50}>100 µg.mL-1 promoted low antioxidant potential (Ngameni et al., 2013). Observing the values for inhibiting DPPH* and ABTS*+ radical it could be noted that all samples of T. guianensis present high antioxidant potential, besides being sources of antioxidant bioactive principles.

In vitro evaluation of anticholinesterase activity

The results found for acetylcholinesterase enzyme inhibition were compared with physostigmine, which is a potent cholinesterase inhibiting alkaloid. It can be observed that three of the four extracts obtained IC_{50} below 20 µgmL-1 (EEFO, EECC and EAFO) while EACC obtained concentration above 20 µg.mL-1. The anticholinesterase potential of extracts and fractions from 54 plants was evaluated and the biological action was classified according to the IC_{50} values as: high potency (IC_{50}<20 µgmL-1); moderate potency (20 < IC_{50}<200 µgmL-1) and low potency (200 < IC_{50}<1000 µgmL-1) (Santos et al., 2018). Through the above classification, we can state that EEFO, EECC, and EAFO samples are considered high potency samples, while EACC obtained moderate potency status.

Several natural products are already reported to have anticholinesterase actions. The actions of 260 compounds against the inhibition of the acetylcholinesterase enzyme were studied. The class of alkaloids was the majority with 139 compounds. Soon after came phenolic compounds with 51 compounds, followed by 18 coumarins, 14 flavonoids, 13 benzenoids, 3 stilbenes, 2 lignans and 1 quinoid (Barbosa-Filho et al., 2008). Rutin and quercetin which were flavonoids detected in the T. guianensis samples of the present study already present reports regarding their cholinesterase potential (Ademosun et al., 2016). In silico studies with the molecules rutin, quercetin and apigenin (also detected in T. guianensis) analyzed the mechanism of action with the enzyme acetylcholinesterase. The study showed molecular interactions of the compounds with active and allosteric sites inhibiting the enzyme action, however only apigenin bound to the active site of the enzyme (Frota et al., 2022). Through these results, one can justify the potential of EEFO, EECC and EAFO in the in vitro anticholinesterase study.

In vitro antifungal activity

Initially, *Tapirira guianensis* extracts were screened for anti-Candida activity against *Candida albincas* strain 0102 (clinical) (Table 3). The aqueous extract of the leaves was the only one that obtained a minimal inhibitory concentration (MIC= 78 µg/ml) and minimal fungicidal concentration (MFC= 625 µg/ml). Thus, the extract was evaluated against a wider spectrum of strains: M1 (clinic); C3 (clinic); 0102 (clinic); 0104 (clinic).

Table 3. Values referring to the Anti-*Candida* potential.

Samples	Strains	MIC(µg/mL)	MFC(µg/mL)
EAFO	M1 (clínica)	9	19
FLZ		1	
EAFO	C3(clínica)	19	39
FLZ		0,25	
EAFO	0102(clínica)	39	78
FLZ		0,25	
EAFO	0104(clínica)	39	78
FLZ		0,25	

MIC: Minimum Inhibitory concentration; MFC Minimum fungicidal concentration; FLZ: Fluconazole and EAFO: aqueous leaf extract. Source: Authors.

Through the table above, it is interesting to emphasize the excellent activity of the aqueous extract of the leaves against all the strains of *C. albicans* tested. This ends up representing a versatility in its application in possible future analyses. In addition, the aqueous extract of the leaves showed the highest content of phenolic compounds and anti-oxidant activity, which may be related to its activity against *Candida*.

Docking Molecular - Evaluation of results *in silico* – Antifugal Activity

The antifungal activity of plant extracts is related to the content of phenolic compounds and their antioxidant potential, because secondary metabolites act in the metabolism of microorganisms (Neves et al., 2022). The aqueous extract of the leaves showed the highest phenolic content and antioxidant potential, thus justifying the best antifungal potential against *Candida albicans* strains. These compounds can induce cell death, rupture the cell membrane, interfere in cell division and development of fungal cells. Among the phenolic compounds that act on microorganisms, we have flavonoids, flavonols and chalcones that act directly on the fungal cell membrane (Seleem, et al., 2017), but there are also tannins that show synergistic action with antibiotics used against microorganisms (Ekambaram, et al., 2016).

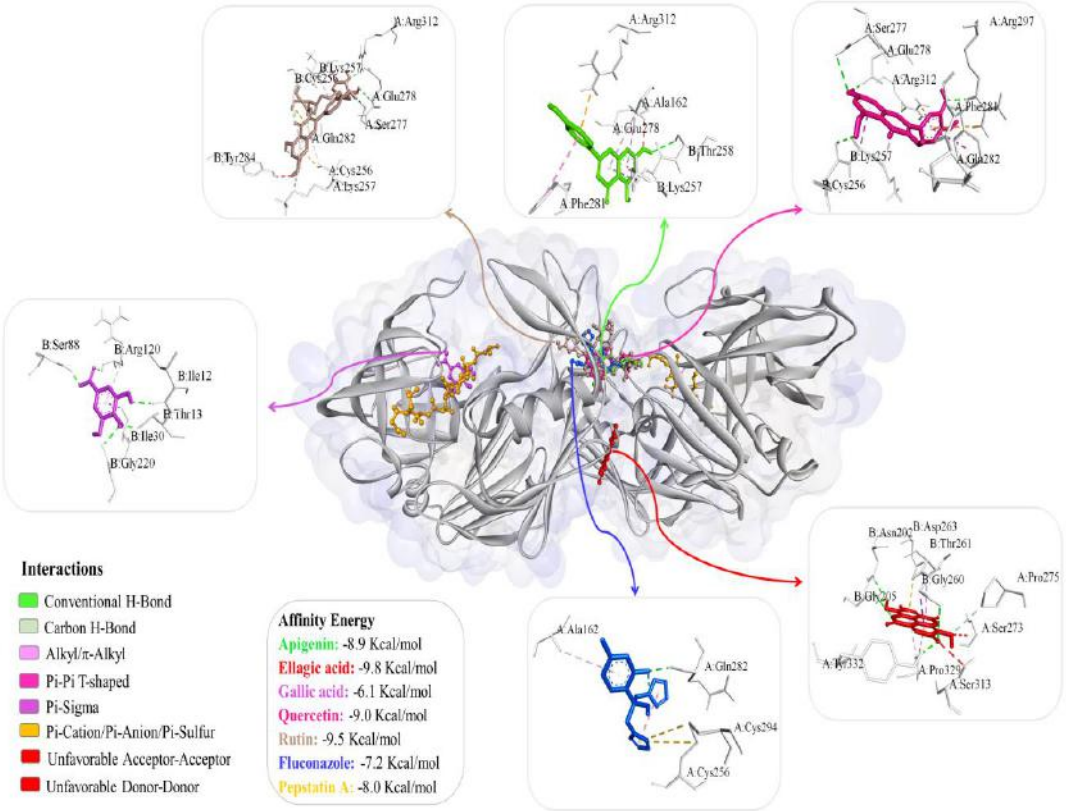
Through the best pose generated we observed RMSD values on the order of 0.012 to 1.790 Å and affinity energy on the order of -6.1 to -9.8 kcal/mol (Table 4). Based on the affinity energy values and compared to fluconazole (-7.2 kcal/mol) we observed that four of the five phytochemicals evaluated showed better affinity energy against SAP5 (Figure 2). The calculated affinity energy for the phytochemicals apigenin and quercetin in this study is respectively -8.9 kcal/mol and -9.0 kcal/mol, while (Meenambiga, et al., 2018) reported values of -5.62 kcal/mol (apigenin) and -5.57 kcal/mol (quercetin), a difference that may be related to differences in the preparation of the receptor and ligand structures before the docking simulations or the exhaustiveness used.

Table 4. Affinity energy and RMSD of the complexes formed with *Candida albicans.*

Receiver	Ligands	*Energy* (kcal mol^{-1})	RMSD (Å)
	Apigenin	-8.9	1.755
	Ellagic acid	-9.8	0.057
	Gallic acid	-6.1	0.290
Candida	Quercetin	-9.0	1.696
albicans	Rutin	-9.5	1.907
	Fluconazole (standard)	-7.2	2.814
	Pep A*	-8.0	1.635

*Ligand co-cristalized (redocking). Source: Authors.

Figure 2. Interaction complex between SAP5, Apigenin (green), Ellagicacid (red), Gallic acid (purple), Quercetin (pink), Rutin (brown), Fluconazole (blue) and the co-crystallized inhibitor Pepstatin A (orange).



Source: Authors.

The figure above demonstrates the interactions of the fluconazole compounds and standard with the protein and visually exposes that apigenin, quercetin and rutin interact in the same region of the fluconazole binding site, thus validating a similar action to the drug.

The analysis of the interaction patterns showed that the SAP5/Apigenin complex showed four Hydrophobic interactions, two of which were with the apolar side chain of the residueAla 162A, two as the basic side chain residue Lys 257B, an H-bondstrong with the uncharged polar side chain residue Thr 258B (2. 96Å), a Pi-Cation interaction as basic side chain residue Arg 312A, a T-shaped Pi-Pi with the apolar side chain of the aromatic residue Phe 281A and an Unfavorable

Acceptor-Acceptor interaction with the acidic side chain residue Glu 278A. The SAP5/Ellagicacid complex showed four Hydrophobic interactions, being two with the apolar side chain of the residue Pro 329A, two with the uncharged polar side chain residues Gly 260B and Thr 261B, five H-bondsstrong interactions with the uncharged polar side chain residues Asn 202B (2. 65 Å), Gly 205B (2.67 and 3.01Å),Gly 260B (2.93 Å), Tyr 332A (2.28 Å), one H-bondweak with the apolar side chain of residuePro 275A (3.68 Å) and two Unfavorable Acceptor-Acceptor interactions with the uncharged polar side chain residues Ser 273A and Ser 313A.

The SAP5/Gallic acid complex showed two Hydrophobic interactions, being one with the apolar side chain of residue Ile 30B, one with the basic side chain residue Arg 120B and five Strong H-bonds, being one with the apolar side chain of residue Ile 12B (2. 20 Å), three with the uncharged polar side chain residues Thr 13B (1.89 Å), Ser 88B (2.16 Å), Gly 220B (2.31 Å) and one with the basic side chain residue Arg 120B (2.36 Å).

The SAP5/Quercetin complex showed two Hydrophobic interactions with the basic side chain residue Lys 257B, three Strong H-bonds, being one with the uncharged polar side chain residue Cys 256B (2.77 Å), one with the basic side chain residue Arg 297A (2.18 Å), one with the acidic side chain residue Glu 278A (1. 82 Å), an H-bond average with the uncharged polar side chain residue Ser 277A (3.05 Å), two Pi-Cation interactions with basic side chain residues Arg 297A and Arg 312A, a T-shaped Pi-Pi with the apolar side chain residue Phe 281A and an Unfavorable Donor-Donor interaction with the uncharged polar side chain residue Gln 282A.

The SAP5/Rutin complex showed three Hydrophobic interactions, two with the uncharged polar side chain residues Cys 256A, Cys 256B, one with the basic side chain residue Lys 257B, six Strong H-bonds, four with the uncharged polar side chain residues Ser 277A (2.49 Å), Gln 282A (2.01, 2.20 and 2.84 Å), one with the basic side chain residue Arg 312A (2. 04 Å), one with the acidic side chain residue Glu 278A(2.83 Å),one H-bond weak with the basic side chain residue Lys 257A (3.78 Å),two Pi-Sulfur interactions with the uncharged polar side chain residues Cys 256A, Cys 256B and one Unfavorable Donor-Donor interaction with the uncharged polar side chain residueTyr 284B.

Table 5 shows the interactions of compounds detected in *T. guianesis* and the fuconazole pattern with SAP5. The SAP5/Fluconazole complex showed two Pi-sulfur interactions with the uncharged polar side chain residues Cys256A and Cys294A, an H-Bond strong with the uncharged polar side chain residue Gln282A (2.25 Å) and a Pi-Alkyl interaction with the apolar side chain of residue Ala162A (Da Silva et al., 2021; Da Silva et al., 2022).

Table 5. Types of interactions and distances (Å) between *Candida albicans* ligands and amino acid residues.

Ligands	Residue	Interaction	Distance (Å)
Apigenin	Ala 162A	Hydrophobic	5.19
	Ala 162A	Hydrophobic	5.42
	Lys 257B	Hydrophobic	4.08
	Lys 257B	Hydrophobic	4.74
	Thr 258B	H-Bond	2.96
	Arg 312A	Pi-Cation	3.81
	Phe 281A	Pi-Pi T-shaped	5.11
	Glu 278A	Unfavorable Acceptor-Acceptor	2.80
Ellagic acid	Gly 260B	Hydrophobic	3.89
	Thr 261B	Hydrophobic	3.85
	Pro 329A	Hydrophobic	5.39
	Pro 329A	Hydrophobic	5.44
	Asn 202B	H-Bond	2.65
	Gly 205B	H-Bond	2.67
	Gly 205B	H-Bond	3.01
	Gly 260B	H-Bond	2.93
	Pro 275A	H-Bond	3.68
	Tyr 332A	H-Bond	2.28
	Asp 263B	Pi-Anion	4.05
	Ser 273A	Unfavorable Acceptor-Acceptor	2.86
	Ser 313A	Unfavorable Acceptor-Acceptor	2.96
Gallic acid	Ile 30B	Hydrophobic	3.84
	Arg 120B	Hydrophobic	5.27
	Ile 12B	H-Bond	2.20
	Thr 13B	H-Bond	1.89
	Ser 88B	H-Bond	2.16
	Arg 120B	H-Bond	2.36
	Gly 220B	H-Bond	2.31
Quercetin	Lys 257B	Hydrophobic	3.57
	Lys 257B	Hydrophobic	4.08
	Cys 256B	H-Bond	2.77
	Ser 277A	H-Bond	3.05
	Glu 278A	H-Bond	1.82
	Arg 297A	H-Bond	2.18
	Arg 297A	Pi-Cation	4.91
	Arg 312A	Pi-Cation	4.27
	Phe 281A	Pi-Pi T-shaped	4.70
	Gln 282A	Unfavorable Donor-Donor	1.46
Rutin	Cys 256A	Hydrophobic	4.66
	Cys 256B	Hydrophobic	5.38
	Lys 257B	Hydrophobic	5.39
	Lys 257A	H-Bond	3.78
	Ser 277A	H-Bond	2.49
	Glu 278A	H-Bond	2.83
	Gln 282A	H-Bond	2.01
	Gln 282A	H-Bond	2.20

	Gln 282A	H-Bond	2.84
	Arg 312A	H-Bond	2.04
	Cys 256A	Pi-Sulfur	4.20
	Cys 256B	Pi-Sulfur	4.07
	Tyr 284B	Unfavorable Donor-Donor	2.59
Fluconazole	Ala 162A	Hydrophobic	5.49
	Gln 282A	H-Bond	2.25
	Cys 256A	Pi-Sulfur	5.78
	Cys 294A	Pi-Sulfur	5.89

Source: Autors.

Through the table above, it is possible to see in detail the types of interaction of the compounds recorded in T. guianensis and fluconazole with the residues of the active site of the SAP5 protein, as well as the interaction distances.

The active site of the SAP5/PepA complex 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, Tyr 225 and Ile 305 (Borelli et al., 2018). Analysis of interactions showed that gallic acid interacts with amino acid residues of the active site of the Sap5/PepA complex (Ile 12B and Gly 220B), indicating similar action to the PepA inhibitor and possibility of a synergistic effect with Fluconazole. Ellagic acid binds at a different site than Pepstatin A and Fluconazole, indicating a possible synergistic effect with the inhibitors. We also observed that the compounds apigenin, quercetin and rutin bind in the same region of the binding site of Fluconazole, having in common interactions with residues Ala 162A (Apigenin), Gln 282A (Quercetin), Cys256A and Gln 282A(Rutin), indicating that these compounds have similar action to Fluconazole.

4. Conclusion

The in vitro studies showed that the aqueous extract of *T. guianensis* leaves has high content of phenolic compounds, thus promoting antioxidant, anti-acetylcholinesterase and antifungal action very promising against *C. albicans*. The molecular docking results showed that the compounds ellagic acid, apigenin, rutin and quercetin showed the best values for affinity energy, however, no significant interactions were observed with the active site of SAP5/PepA complex, indicating that these compounds interact with other sites of SAP5.

Although the affinity energy of gallic acid (-6.1 kcal/mol) was higher than the affinity energy of the aspartic protease inhibitor Pepstatin A (-8.0 kcal/mol), gallic acid interacts with residues in the active site of the SAP5/PepA complex, indicating a similar effect to the PepstatinA inhibitor. Therefore, the present study suggests the use of gallic acid as a model to generate new molecules that may have better affinity energy and significant interactions with SAP5, thus gallic acid shows promise in the planning and development of new antifungal drugs acting against the secreted aspartic Proteinase (SAP5) of *Candida albicans*.

The present research proposes to the scientific community studies of isolation, purification, and characterization of active principles from the species Tapirira guianensis, thus projecting new therapeutic proposals against Alzheimer's disease and Candidiasis (the latter which has become a global public health problem).

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References

Ademosun, A. O., Oboh, G., Bello, F., & Ayeni, P. O. (2016). Antioxidative Properties and Effect of Quercetin and Its Glycosylated Form (Rutin) on Acetylcholinesterase and Butyrylcholinesterase Activities. *Journal of evidence-based complementary & alternative medicine*, *21*(4), 11-17. https://doi.org/10.1177/2156587215610032

Antonopoulou, I., Sapountzaki, E., Rova, U., & Christakopoulos, P. (2022). The Inhibitory Potential of Ferulic Acid Derivatives against the SARS-CoV-2 Main Protease: Molecular Docking, Molecular Dynamics, and ADMET Evaluation. *Biomedicines*, *10*(8), 1787. https://doi.org/10.3390/biomedicines10081787

Araújo, C. R. M., Santos, V. L. dos A., & Arlan, A. G. (2016). Acetilcolinesterase - AChE: Uma Enzima de Interesse Farmacológico. *Revista Virtual de Química*, *8*(6), 1818–1834

Badhani, B., Sharma, N., & Kakkar, R. (2015). Ácido gálico: um antioxidante versátil com aplicações terapêuticas e industriais promissoras. *Rsc Advances*, *5* (35), 27540-27557. https://doi.org/10.1039/C5RA01911G

Barbosa, F. G., Lima, M. A. S.., Braz-Filho, R., & Silveira, E R. (2006). Glicosídeos iridóides e feniletanoides de Lippia alba. *Biochemical Systematics and Ecology*, *11* (34), 819-821. https://doi.org/10.1016/j.bse.2006.06.006

Becker, M., Nunes, G., Ribeiro, D., Silva, F., Catanante, G., & Marty, J. (2019). Determination of the Antioxidant Capacity of Red Fruits by Miniaturized Spectrophotometry Assays. *Journal of the Brazilian Chemical Society*, *3*(4), 223–227. https://doi.org/10.21577/0103-5053.20190003

Biovia, D. S., Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., & Richmond, T. J. (2000). Dassault Systèmes BIOVIA, Discovery Studio Visualizer. *The Journal of Chemical Physics*, *17*(2).

Borelli, C., Ruge, E., Lee, J. H., Schaller, M., Vogelsang, A., Monod, M., & Maskos, K. (2008). X-ray structures of Sap1 and Sap5: structural comparison of the secreted aspartic proteinases from *Candida albicans*. *Proteins: Structure, Function, and Bioinformatics*, *72*(4), 1308-1319. https://doi.org/10.1002/prot.22021

Bors, W., & Saran, M. (1987). Radical scavenging by flavonoid antioxidants. *Free radical research communications*, *2*(4-6), 289-294. https://doi.org/10.3109/10715768709065294

Clinical and Laboratory Standards Institute (CLSI, formerly National Commitee for Clinical and Laboratory Standards NCCLS) (2018). Method M-38ª, (2ª ed), Wayne, Ed.; NCCLS Pennsylvania, *22* (16) 1-27

Correia, S. D. J., DavidI, J. M., Silva, E. P. D., David, J. P., Lopes, L. M., & Guedes, M. L. S. (2008). Flavonóides, norisoprenóides e outros terpenos das folhas de *Tapirira guianensis* . *Química Nova*, *31*, 2056-2059. https://doi.org/10.1590/S0100-40422008000800027

Csizmadia, P. (1999) In *Proceedings of The 3rd International Electronic Conference on Synthetic Organic Chemistry*; MDPI: Basel, Switzerland, 1775.

da Silva, C. R., Moura, F. L. D., Alves Filho, A. L. A., de Sousa Campos, R., de Farias Cabral, V. P., Sá, L. G. D. A. V., & Neto, J. B. D. A. (2022). Evaluation of interactions of silibinin with the proteins ALS3 and SAP5 against *Candida albicans*. *Journal of Health & Biological Sciences*, *10*(1), 1-6. http://dx.doi.org/10.12662/2317-3076jhbs.v10i1.4239.p1-6.2022

da Silva, L. J., Barroso, F. D. D., Vieira, L. S., Mota, D. R. C., da Silva Firmino, B. K., da Silva, C. R., & de Andrade Neto, J. B. (2021). Diazepam's antifungal activity in fluconazole-resistant Candida spp. and biofilm inhibition in C. albicans: evaluation of the relationship with the proteins ALS3 and SAP5. *Journal of Medical Microbiology*, *70*(3), 001308. https://doi.org/10.1099/jmm.0.001308

D'Andrea, G. (2015). Quercetin: a flavonol with multifaceted therapeutic applications?. *Fitoterapia*, *106*, 256-271. https://doi.org/10.1016/j.fitote.2015.09.018

David, J. M., Chávez, J. P., Chai, H. B., Pezzuto, J. M., & Cordell, G. A. (1998). Two new cytotoxic compounds from *Tapirira guianensis* . *Journal of natural products*, *61*(2), 287-289. https://doi.org/10.1021/np970422v

de Andrade Neto, J. B., de Farias Cabral, V. P., Nogueira, L. F. B., da Silva, C. R., Sa, L. G. D. A. V., da Silva, A. R., & Júnior, H. V. N. (2021). Anti-MRSA activity of curcumin in planktonic cells and biofilms and determination of possible action mechanisms. *Microbial pathogenesis*, *155*, 104892. https://doi.org/10.1016/j.micpath.2021.104892

de Morais, S. M., da Silva Lopes, F. F., Fontenele, G. A., da Silva, M. V. F., Fernandes, V. B., & Alves, D. R. (2021). Total phenolic content and antioxidant and anticholinesterase activities of medicinal plants from the State's Cocó Park (Fortaleza-CE, Brazil). *Research, Society and Development*, *10*(5), e7510514493-e7510514493. https://doi.org/10.33448/rsd-v10i5.14493

Duthie, G. G., Duthie, S. J., & Kyle, J. A. (2000). Plant polyphenols in cancer and heart disease: implications as nutritiona1 antioxidants. *Nutrition research reviews*, *13*(1), 79-106. https://doi.org/10.1079/095442200108729016

Ekambaram, S. P., Perumal, S. S., & Balakrishnan, A.(2016). *Phytotherapy research 30*, 1035. https://doi.org/10.1002/ptr.5616

Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*(2), 88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Fontenelle, R. O. S., Morais, S. M., Brito, E. H. S., Kerntopf, M. R., Brilhante, R. S. N., Cordeiro, R. A., & Rocha, M. F. G. (2007). Chemical composition, toxicological aspects and antifungal activity of essential oil from Lippia sidoides Cham. *Journal of Antimicrobial Chemotherapy*, *59*(5), 934-940. https://doi.org/10.1093/jac/dkm066

Frota, L. S., Lopes, F. F. S., Alves, D. R., Freitas, L. S., Franco, G. M. G., & Morais, S. M. de. (2021). Composição química e avaliação das atividades

antioxidante e anticolinesterásica do óleo dos frutos de Ouratea fieldingiana (Gargner) Engl. *Research, Society and Development*, *10*(10), e532101019013. https://doi.org/10.33448/rsd-v10i10.19013

Funari, C. S., & Ferro, V. O. (2006). Análise de própolis. *Food Science and Technology*, *26*, 171-178. https://doi.org/10.1590/S0101-20612006000100028

Gupta, A., Kumar, R., Ganguly, R., Singh, A. K., Rana, H. K., & Pandey, A. K. (2021). Antioxidant, anti-inflammatory and hepatoprotective activities of Terminalia bellirica and its bioactive component ellagic acid against diclofenac induced oxidative stress and hepatotoxicity. *Toxicology reports*, *8*, 44-52. https://doi.org/10.1016/j.toxrep.2020.12.010

Halgren, T. A. (1996). Merck molecular force field. II. MMFF94 van der Waals and electrostatic parameters for intermolecular interactions. *Journal of Computational Chemistry*, *17*(5–6), 520–552. https://doi.org/10.1002/(SICI)1096-987X(199604)17:5/6<520::AID-JCC2>3.0.CO;2-W

Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. (2012). Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics*, *4*(1), 17. https://doi.org/10.1186/1758-2946-4-17

Huey, R., Morris, G. M., & Forli, S. (2012). Using AutoDock 4 and AutoDock vina with AutoDockTools: a tutorial. *The Scripps Research Institute Molecular Graphics Laboratory*, *10550*, 92037.

Imani, A., Maleki, N., Bohlouli, S., Kouhsoltani, M., Sharifi, S., & Maleki Dizaj, S. (2021). Molecular mechanisms of anticancer effect of rutin. *Phytotherapy Research*, *35*(5), 2500-2513. https://doi.org/10.1002/ptr.6977

Imberty, A., Hardman, K. D., Carver, J. P., & Perez, S. (1991). Molecular modelling of protein-carbohydrate interactions. Docking of monosaccharides in the binding site of concanavalin A. *Glycobiology*, *1*(6), 631-642. https://doi.org/10.1093/glycob/1.6.631

Ivanov, M., Kannan, A., Stojković, D. S., Glamočlija, J., Calhelha, R. C., Ferreira, I. C., & Soković, M. (2020). Flavones, flavonols, and glycosylated derivatives impact on *Candida albicans* growth and virulence, expression of cdr1 and erg11, cytotoxicity. *Pharmaceuticals*, *14*(1), 27. https://doi.org/10.3390/ph14010027

Janeczko, M., Gmur, D., Kochanowicz, E., Górka, K., & Skrzypek, T. (2022). Inhibitory effect of a combination of baicalein and quercetin flavonoids against *Candida albicans* strains isolated from the female reproductive system. *Fungal Biology*, *126*(6-7), 407-420. https://doi.org/10.1016/j.funbio.2022.05.002

Kim, G. H., Kim, J. E., Rhie, S. J., & Yoon, S. (2015). The role of oxidative stress in neurodegenerative diseases. Experimental neurobiology, 24(4), 325.

Kwun, M. S., & Lee, D. G. (2020). Quercetin-induced yeast apoptosis through mitochondrial dysfunction under the accumulation of magnesium in Candida albicans. *Fungal biology*, *124*(2), 83-90. https://doi.org/10.1016/j.funbio.2019.11.009

Li, Z. J., Liu, M., Dawuti, G., Dou, Q., Ma, Y., Liu, H. G., & Aibai, S. (2017). Antifungal activity of gallic acid in vitro and in vivo. *Phytotherapy research*, *31*(7), 1039-1045. https://doi.org/10.1002/ptr.5823

Marinho, E. M., de Andrade Neto, J. B., Silva, J., da Silva, C. R., Cavalcanti, B. C., Marinho, E. S., & Júnior, H. V. N. (2020). Virtual screening based on molecular docking of possible inhibitors of Covid-19 main protease. *Microbial Pathogenesis*, *148*, 104365. https://doi.org/10.1016/j.micpath.2020.104365

Meenambiga, S. S., Venkataraghavan, R., & Biswal, R. A. (2018). In silico analysis of plant phytochemicals against secreted aspartic proteinase enzyme of *Candida albicans*. *Journal of Applied Pharmaceutical Science*, *8*(11), 140-150.

Naves, P. L. F., Santana, D. P., Ribeiro, E. L., & Menezes, A. C. S. (2013). Novas abordagens sobre os fatores de virulência de *Candida albicans*. *Revista de Ciências Médicas e Biológicas*, *12*(2), 229-233. https://doi.org/10.9771/cmbio.v12i2.6953

Neves, A. M., Morais, S. M. D., Santos, H. S. D., Ferreira, M. M., Cruz, R. C. V., Souza, E. B. D., & Fontenelle, R. O. D. S. (2022). Prospecção química, atividade antioxidante, anticolinesterásica e antifúngica de extratos etanólicos de espécies de Senna Mill.(Fabaceae). *Hoehnea*, *49*. https://doi.org/10.1590/2236-8906-111/2020

Ngameni, B., Fotso, G. W., Kamga, J., Ambassa, P., Abdou, T., Fankam, A. G., & Kuete, V. (2013). Flavonoids and Related Compounds from the Medicinal Plants of Africa. In *Medicinal Plant Research in Africa* (pp. 301–350). Elsevier. https://doi.org/10.1016/B978-0-12-405927-6.00009-6

Ohno, Y., Fukuda, K., Takemura, G., Toyota, M., Watanabe, M., Yasuda, N., & Fujiwara, H. (1999). Induction of apoptosis by gallic acid in lung cancer cells. *Anti-cancer drugs*, *10*(9), 845-851. https://doi.org/10.1097/00001813-199910000-00008

Patient, A., Jean-Marie, E., Robinson, J. C., Martial, K., Meudec, E., Levalois-Grützmacher, J., & Bereau, D. (2022). Polyphenol Composition and Antioxidant Activity of *Tapirira guianensis* Aubl.(Anarcadiaceae) Leaves. *Plants*, *11*(3), 326. https://doi.org/10.3390/plants11030326

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera - A visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, *25*(13), 1605–1612. https://doi.org/10.1002/jcc.20084

Raina, K., Rajamanickam, S., Deep, G., Singh, M., Agarwal, R., & Agarwal, C. (2008). Chemopreventive effects of oral gallic acid feeding on tumor growth and progression in TRAMP mice. *Molecular cancer therapeutics*, *7*(5), 1258-1267. https://doi.org/10.1158/1535-7163.MCT-07-2220

Rao, A. S., & Camilleri, M. (2010). metoclopramide and tardive dyskinesia. *Alimentary pharmacology & therapeutics*, *31*(1), 11-19. https://doi.org/10.1111/j.1365-2036.2009.04189.x

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Ren, L., Zhang, J., & Zhang, T. (2021). Immunomodulatory activities of polysaccharides from Ganoderma on immune effector cells. *Food Chemistry*, *340*, 127933. https://doi.org/10.1016/j.foodchem.2020.127933

Rodrigues, A. M., Guimarães, D. O., Konno, T. U., Tinoco, L. W., Barth, T., Aguiar, F. A., & Muzitano, M. F. (2017). Phytochemical study of *Tapirira guianensis* leaves guided by vasodilatory and antioxidant activities. *Molecules*, *22*(2), 304. https://doi.org/10.3390/molecules22020304

Roumy, V., Fabre, N., Portet, B., Bourdy, G., Acebey, L., Vigor, C., & Moulis, C. (2009). Four anti-protozoal and anti-bacterial compounds from *Tapirira guianensis* . *Phytochemistry*, *70*(2), 305-311. https://doi.org/10.1016/j.phytochem.2008.10.003

Santos, T. C. D., Gomes, T. M., Pinto, B. A. S., Camara, A. L., & Paes, A. M. D. A. (2018). Naturally occurring acetylcholinesterase inhibitors and their potential use for Alzheimer's disease therapy. *Frontiers in Pharmacology*, *9*, 1192. https://doi.org/10.3389/fphar.2018.01192

Schelenz, S. (2008). Management of candidiasis in the intensive care unit. Journal of Antimicrobial Chemotherapy, 61(suppl_1), i31-i34.

Schrödinger, L. L. C. The PyMOL Molecular Graphics System, Version 2.3. 2019. *Google Scholar There is no corresponding record for this reference*.

Seleem, D., Pardi, V., & Murata, R. M. (2017). Review of flavonoids: A diverse group of natural compounds with anti-*Candida albicans* activity in vitro. *Archives of oral biology*, *76*, 76-83. https://doi.org/10.1016/j.archoralbio.2016.08.030

Sharma, A., Kashyap, D., Sak, K., Tuli, H. S., & Sharma, A. K. (2018). Therapeutic charm of quercetin and its derivatives: a review of research and patents. *Pharmaceutical patent analyst*, *7*(1), 15-32. https://doi.org/10.4155/ppa-2017-0030

Shityakov, S., & Förster, C. (2014). In silico predictive model to determine vector-mediated transport properties for the blood–brain barrier choline transporter. *Advances and applications in bioinformatics and chemistry: AABC*, *7*, 23. https://doi.org/10.2147/AABC.S63749

Silva, E. P. D., David, J. M., David, J. P., Garcia, G. H. T., & Silva, M. T. (2020). Chemical composition of biological active extracts of *Tapirira guianensis* (Anacardiaceae). *Química Nova*, *43*, 1216-1219. https://doi.org/10.21577/0100-4042.20170605

Silva, J., Rocha, M. N., & Marinho, E. M. (2021). Evaluation of the ADME, toxicological analysis and molecular docking studies of the anacardic acid derivatives with potential antibacterial effects against staphylococcus aureus. *J Anal Pharm Res*, *10*(5), 177-194. https://doi.org/10.15406/japlr.2021.10.00384

Silva-Oliveira, R. J., Lopes, G. F., Camargos, L. F., Ribeiro, A. M., Santos, F. V. D., Severino, R. P., & Ribeiro, R. I. M. D. A. (2016). *Tapirira guianensis* Aubl. extracts inhibit proliferation and migration of oral cancer cells lines. *International journal of molecular sciences*, *17*(11), 1839. https://doi.org/10.3390/ijms17111839

Singh, D., Khan, M. A., Akhtar, K., Arjmand, F., & Siddique, H. R. (2022). Apigenin alleviates cancer drug Sorafenib induced multiple toxic effects in Swiss albino mice via anti-oxidative stress. *Toxicology and Applied Pharmacology*, *447*, 116072. https://doi.org/10.1016/j.taap.2022.116072

Smiljkovic, M., Stanisavljevic, D., Stojkovic, D., Petrovic, I., Vicentic, J. M., Popovic, J., & Sokovic, M. (2017). Apigenin-7-O-glucoside versus apigenin: Insight into the modes of anticandidal and cytotoxic actions. *EXCLI journal*, *16*, 795. https://doi.org/10.17179/excli2017-300

Sousa, C. M. D. M., Silva, H. R., Ayres, M. C. C., Costa, C. L. S. D., Araújo, D. S., Cavalcante, L. C. D., & Chaves, M. H. (2007). Fenóis totais e atividade antioxidante de cinco plantas medicinais. *Química nova*, *30*, 351-355. https://doi.org/10.1590/S0100-40422007000200021

Trott, O., & Olson, A. J. (2009). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461. https://doi.org/10.1002/jcc.21334

Trubiano, J. A., & Padiglione, A. A. (2015). Nosocomial infections in the intensive care unit. Anaesthesia & Intensive Care Medicine, 16(12), 598-602.

Wang, W., Sun, C., Mao, L., Ma, P., Liu, F., Yang, J., & Gao, Y. (2016). The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. *Trends in Food Science & Technology*, *56*, 21-38. https://doi.org/10.1016/j.tifs.2016.07.004

Yan, J., Zhang, G., Pan, J., & Wang, Y. (2014). α-Glucosidase inhibition by luteolin: Kinetics, interaction and molecular docking. *International journal of biological macromolecules*, *64*, 213-223. https://doi.org/10.1016/j.ijbiomac.2013.12.007

Yan, X., Qi, M., Li, P., Zhan, Y., & Shao, H. (2017). Apigenin in cancer therapy: Anti-cancer effects and mechanisms of action. *Cell & Bioscience*, *7*(1), 1-16. https://doi.org/10.1186/s13578-017-0179-x

Yang, J., Guo, J., & Yuan, J. (2008). In vitro antioxidant properties of rutin. *LWT-Food Science and Technology*, *41*(6), 1060-1066. https://doi.org/10.1016/j.lwt.2007.06.010

Yusuf, D., Davis, A. M., Kleywegt, G. J., & Schmitt, S. (2008). An alternative method for the evaluation of docking performance: RSR vs RMSD. *Journal of chemical information and modeling*, *48*(7), 1411-1422. https://doi.org/10.1021/ci800084x

Zhang, T., Zhong, S., Li, T., & Zhang, J. (2020). Saponins as modulators of nuclear receptors. *Critical reviews in food science and nutrition*, *60*(1), 94-107. https://doi.org/10.1080/10408398.2018.1514580