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Chemistry

Polyphenols of four medicinal plant extracts and relation with antifungal activities through *in vitro* and *in silico* studies

Polifenóis de quatro extratos de plantas medicinais e relação com atividades antifúngicas por meio de estudos *in vitro* e *in sílico*

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ABSTRACT

Several medicinal plant extracts contain phenolic compounds with antifungal properties that are useful in pharmaceutical formulations. This study selected species from Cocó River State Park in Fortaleza, Ceará, Brazil, and compared their efficiency as antifungal products. To achieve this goal, the phenolic profile and anticandidal actions of extracts were evaluated, and the main constituents were characterized and correlated with antifungal properties through *in vitro* and *in silico* studies. *Anacardium occidentale*, *Myracrodruon urundeuva, Laguncularia racemosa*, and *Terminalia catappa* were chosen. The plant parts were collected using folk medicine recommendations. The main compounds present in the extracts were gallic acid, epicatechin, ellagic acid, isoquercitrin, quercetin and rutin, detected by high performance liquid chromatography analysis. The anticandidal activity of extracts varied from high to moderate, and *A. occidentale* presented the best activity, followed by *L. racemosa*. The *in silico* studies revealed that affinity energy (for ellagic acid (-9.4), isoquercitrin (-9.3), and rutin (-9.0) kcal moL^{-1} were better to secreted aspartic proteinase 5 (Sap5) from *Candida albicans.* Nevertheless, ellagic acid and isoquercitrin act in different places in relation to the active site of Sap5 and could synergize with fluconazole.

Keywords: Medicinal plants; Polyphenols; HPLC-DAD; Molecular Docking; Candida albicans

RESUMO

Vários extratos de plantas medicinais contêm compostos fenólicos com propriedades antifúngicas úteis em formulações farmacêuticas. Este estudo selecionou espécies do Parque Estadual do Rio Cocó em

Fortaleza, Ceará, Brasil, e comparou sua eficiência como antifúngico. Para atingir este objetivo foram avaliados o perfil fenólico e as ações anticandidas dos extratos, os principais constituintes foram caracterizados e correlacionados com as propriedades antifúngicas através de estudos *in vitro* e *in silico*. *Anacardium occidentale, Myracrodruon urundeuva, Laguncularia racemosa* e *Terminalia catappa* foram escolhidas. As partes das plantas foram coletadas de acordo com a recomendação da medicina popular. Os principais compostos presentes nos extratos foram ácido gálico, epicatequina, ácido elágico, isoquercitrina, quercetina e rutina, detectados por cromatografia líquida de alta eficiência. A atividade anticandida dos extratos variou de alta a moderada, sendo que *A. occidentale* apresentou a melhor atividade seguida por *L. racemosa*. Os estudos *in silico* revelaram que a energia de afinidade (ΔG) para ácido elágico (-9,4), isoquercitrina (-9,3) e rutina (-9,0) kcal moL^{-1} foram melhores em relação à proteinase aspártica 5 secretada (Sap5) de *Candida albicans*, porém o ácido elágico e a isoquercitrina atuam em locais diferentes em relação ao sítio ativo do Sap5 e poderiam atuar em sinergismo com o fluconazol.

Palavras-chave: Plantas medicinais; Polifenóis; HPLC-DAD; Docking Molecular; Candida albicans

1 INTRODUCTION

One of the main representatives of plants' secondary metabolism is phenolic compounds, which are considered responsible for numerous pharmacological activities, including antioxidant and antifungal activities. They can be classified as simple phenols or polyphenols, and their main representatives are phenolic acids and flavonoids (Lima Neto et al., 2015; Neves et al., 2022).

The effects of phenolic compounds on human health have been well described, but the actual mechanism is not yet well explained. One action may be the redox homeostasis regulation by scavenging or generating reactive oxygen species (ROS). ROS are better known for causing cytotoxicity but can also affect various cellular signal cascades and metabolic processes. ROS are required in specific low concentrations for normal cell functioning, including cell defense against microorganisms. Many plant secondary metabolites act as antioxidants and pro-oxidants and can affect ROS concentrations, depending on the reaction conditions (Kessler et al., 2010; Teodoro et al., 2015). The pro-oxidant action could be assessed to combat fungal infections, including Candidiasis.

In healthy hosts, the first line of defense against *Candida albicans* is through phagocytosis by innate immune cells, including macrophages and neutrophils. A major

antimicrobial defense mechanism of these phagocytes is the production of reactive oxygen species (ROS) through the respiratory chain (Chobot and Hadacek, 2011). So, the balance of antioxidant and pro-oxidant flavonoid activities can combat these microorganisms.

The incidence of fungal infections is rising, mainly due to growing resistance to conventional antifungal drugs. The indiscriminate use of these drugs against various diseases has contributed to the emergence of antimicrobial resistance, representing a substantial challenge for the global health community. This phenomenon is a persistent threat to public health, with significant social and financial implications. Given this scenario, the search for new compounds as therapeutic alternatives has become imperative. Substances obtained from medicinal plants have emerged as a valuable source in developing antimicrobial agents, offering promising prospects for tackling this emerging challenge (Viana et al., 2022).

Identifying flavonoids with possible antifungal effects at small concentrations or synergistic combinations can help overcome this problem. Flavonoids found in plant parts, including fruits and seeds, have anti-inflammatory, antimutagenic, anticancer, and antioxidant properties, so they are of interest for the production of pharmaceuticals, nutraceuticals, and cosmetics (Al Aboody & Mickymaray, 2020; Frota et al., 2022).

Proteolytic activity is an important virulence factor for *C. albicans*. This is attributed to the family of secreted aspartic proteinases (Saps) from *C. albicans*, with a minimum of 10 members. Saps show controlled expression and regulation for the individual stages of the infection process. Design of inhibitors specific to Sap5 seems to play a major part in fighting superficial Candida infections (Borelli et al., 2008).

Previous studies of medicinal plants from Cocó River State Park in Fortaleza, Ceará, Brazil, revealed species with a high content of phenolic compounds (Morais et al., 2021) as *Anacardium occidentale,* which displays antimicrobial activity verified in Gram-positive and negative bacteria and fungi (Araújo et al., 2020). *Myracrodrum urundeuva* bark extracts showed potential for topical treatment of infections caused by some Candida

species (Oliveira et al., 2017), both from the Anacardiaceae family. Two Combretaceae plants also display antifungal actions: *Terminalia catappa* leaves were tested on Candida reference strains and clinical isolates from patients with acquired immunodeficiency syndrome (AIDS) (Terças et al., 2017) and the ethyl acetate extracts of 70 endophytic fungi strains, isolated from leaves of Brazilian mangrove plant *Laguncularia racemosa* showed action towards several bacteria (Silva et al., 2011).

Despite the uses previously attributed to these species, few studies have been conducted, leaving a vast area to be explored. From this perspective, research into their fungicidal properties is of notable importance, with a view to bioprospecting and developing bioproducts with pharmacological applications. Therefore, this study aimed to analyze the anti-candida properties present in the ethanolic extracts of four medicinal plants collected from the medicinal flora of the Cocó State Park (Fortaleza, Ceará, Brazil). In addition, it was sought to evaluate the antioxidant profile, the phenolic composition using HPLC-DAD analysis, and the antifungal activities against *C. albicans* yeast strains. In addition, a computational study of the mechanism of action was carried out, considering the main compounds characterized concerning the macromolecular target called secreted aspartic proteinase 5 (Sap5).

2 MATERIAL AND METHODS

2.1 Chemicals and reagents

To carry out the tests here, solvents from J.T. Baker – Radnor, USA; Exodo Cientifica - São Paulo, BR; and Neon - São Paulo, Brazil were used. The reagents were purchased from Sigma-Aldrich – St. Louis, USA and Carvalhães – Alvorada, RS, Brazil. The device used was a Shimadzu high-performance liquid chromatograph - Kyoto, Japan. Analytical standards (ellagic acid, epicatechin, gallic acid, isoquercitrin, rutin and quercetin) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Chemicals and reagents

The plant material was collected from a garden of the Cocó State Park, Fortaleza, Ceará, Brazil. Exsiccates were identified by the botanist Luiz Wilson Lima-Verde and deposited in the Prisco Bezerra Herbarium of the Federal University of Ceará (UFC). The license for the collection of plant material was granted by the State Secretariat for the Environment of Ceará through authorization 29/2019. Data for each species are listed in Table 1.

Table 1 – As tabelas devem ter seus títulos na parte superior

Species	Family	Part used	Exsicate	Coordinators
Anacardium occidentale Linnaeus	Anacardiaceae	Stem bark	63659	3°44'41.5"S 38°29'10.2"W
Myracrodruon urundeuva M. Allemão	Anacardiaceae	Stem bark	62695	3°44'39.3"S 38°29'11.9"W
Laguncularia racemosa (L.) C. F. Gaertn.	Combretaceae	Leaves	38597	3°44'55.3"S 38°29'04.8"W
Terminalia catappa Linnaeus	Combretaceae	Leaves	63657	3°44'52.3"S 38°29'05.9"W

2.3 Chemicals and reagents

Samples of each plant (leaves or stem bark) were dried in an oven with air circulation for 6 hours at 60 ºC and subsequently processed in a knife mill. Then, 50 g of each plant material was macerated in 200 mL of ethanol for 10 days, and the resulting solution was filtered and concentrated with a rotary evaporator under vacuum pressure at 60 °C. After this process, the material was left in a water bath at 40 °C until the solvent was eliminated to obtain each species' extract.

2.4 Characterization of phenolic compounds by HPLC in extracts

The identification and quantification of phenolic compounds were performed using a Shimadzu high-performance liquid chromatography (HPLC) and Shimadzu Prominence

auto sampler (SIL-20A) integrated with an SPD-M20A diode array detector (DAD) using the LC Solution 1.22 SP1 software. Analyses were performed with Shim-Pack (CLC) ODS Gold reversed-phase column (5 µm; 4.6 x 250 mm). The mobile phase comprised a mixture of solvent A (acetonitrile) and solvent B (Milli-Q water acidified to pH 2.8 with phosphoric acid). The elution gradient was programmed at 0-15 min, 20% A, 80% B; 17-25 min, 40% A, 60% B; 25-40 min, 20% A, 80% B. Flow rate of 1 mL mim^{-1}, injection volume of 20 µL, column temperature of 40 °C and wavelength from 350 nm were used. The chromatographic peaks were confirmed by comparing their retention time with that of the reference standard and by UV spectra in DAD. Samples were analyzed in triplicate. The average areas under the peaks were measured to quantify the compounds through the calibration curve built with the standards. The method's calibration curves were obtained by plotting the peak area (counts) versus the concentration of the standards (mg mL^{-1}). All calibration points were chosen to be five. The concentration range varied from 0.001 to 1.0 mg mL^{-1} for all analytes. In the validation of the analytical method, the system parameters adequacy, specificity, linearity, detection limit and quantification limit were evaluated in accordance with the International Guidelines of the Conference on Harmonization (ICH) Q2 R1 (Dimcheva et al., 2019).

2.5 Determination of antifungal activity

Antifungal activity was measured according to the method described by Fontenelle et al. (2007) using broth microdilution MIC tests based on the Clinical Laboratory Standards Institute protocol M38-A (2018). C. albicans fungal strains (ATCC, 0102, 0104 and 0105) obtained from the mycoteca of the Federal University of Pernambuco were used. The MIC was determined in 96-well microplates in which 10 mg mL^{-1} of diluted extract (50 µL of 5% DMSO and 950 µL of RPMI medium) and 50 µL of RPMI medium were added to all wells of the first column, followed by the addition of a series of dilutions (0.002 to 2.5 mg mL^{-1}) and 100 µL of the inoculum. The

plates were incubated at 37 °C, and visual reading was performed after 48 hours. The positive control used was fluconazole. The assays were performed in duplicate, and the MIC was defined as the lowest sample concentration capable of inhibiting 100% of the visible growth of the microorganism. Results were determined by visualization as recommended by CLSI.

2.6 Molecular docking

2.6.1 Ligand preparation and optimization

The chemical structures of the ligands ellagic acid, epicatechin, gallic acid, isoquercitrin, rutin, quercetin and fluconazole were obtained from the PubChem database. The Avogadro code optimized the structures by applying the MMFF94 force field (Merck Molecular Force Field 94) and the “steepest descent” algorithm with 50 cycles of interactions from the Marvin Sketch™ and Avogadro™ codes. All ligands were evaluated separately in molecular studies (ChemAxon, 1998; Csizmadia, 1999; Hanwell et al., 2012).

2.6.2 Obtaining the 3D structure of the target protein

The structure of the target macromolecules was obtained from the Protein Data Bank repository, identified as “Secreted aspartic proteinase (Sap) 5 from Candida albicans”, deposited with the code (PDB: 2QZX) generated from X-ray diffraction (R-Value Free: 0.275 and R-Value Work: 0.224) with resolution of 2.50 Å, classified as hydrolase expressed in Escherichia coli system and as Transferase, organism C. albicans (Dastmalchi et al., 2016).

2.6.3 General preparation and procedures

The Sap5 commonly used to assess anti-candida activity was prepared by removing residues present in the protein, following additional charges of polar hydrogen

and Gasteiger through the AutoDock Tools ™ software (Apache, 2007; Silva et al., 2022; Yan et al., 2014). Finally, 50 independent molecular docking simulations were performed for each ligand using the Lamarckian Genetic Algorithm (LGA) and Exhaustiveness 64 using the AutoDock Vina software.

Two criteria were used to select the best pose. The first was the root mean square deviation (RMSD), a validation criterion for simulations performed with ideal parameters up to 2.0 Å (Dastmalchi et al., 2016). The second criterion used was affinity energy (ΔG), considered ideal when presenting values less than or equal to -6.0 kcal mol^{-1} (Shityakov and Foerster, 2014). The selectivity of the ligand against the *C. albicans* target was determined by the inhibition constant (Ki) of the receptor-ligand complexes formed, calculated through ΔG (Kadela-tomanek et al., 2021).

The strength of the hydrogen bond (H-bond) based on the values of the distances between the donor and acceptor atoms was classified as strong for distances between 2.5-3.1 Å, moderate for distances between 3.1-3.55 Å and weak for distances greater than 3.55 Å (Imberty et al., 1991).

3 RESULTS AND DISCUSSION

3.1 Characterization of phenolic compounds

Flavonoids can inhibit fungal growth through various mechanisms, including plasma membrane disruption, the induction of mitochondrial dysfunction, and inhibiting cell wall formation, cell division, RNA and protein synthesis, and the efflux-mediated pumping system. It is possible that flavonoid prooxidant function could take a toll on their fungicidal or bactericidal functions. For example, epigallocatechin gallate promotes apoptosis and bactericidal activity, attributed to its ability to reduce O_2 to yield H_2O_2 (Maeta et al., 2007).

The phenolic compounds characterized by high-performance liquid chromatography (HPLC) analysis were gallic acid, isoquercitrin, ellagic acid, epicatechin, quercetin and rutin (Figure 1).

Figure 1 – Chemical representation of the phenolic compound's structures detected in the extracts

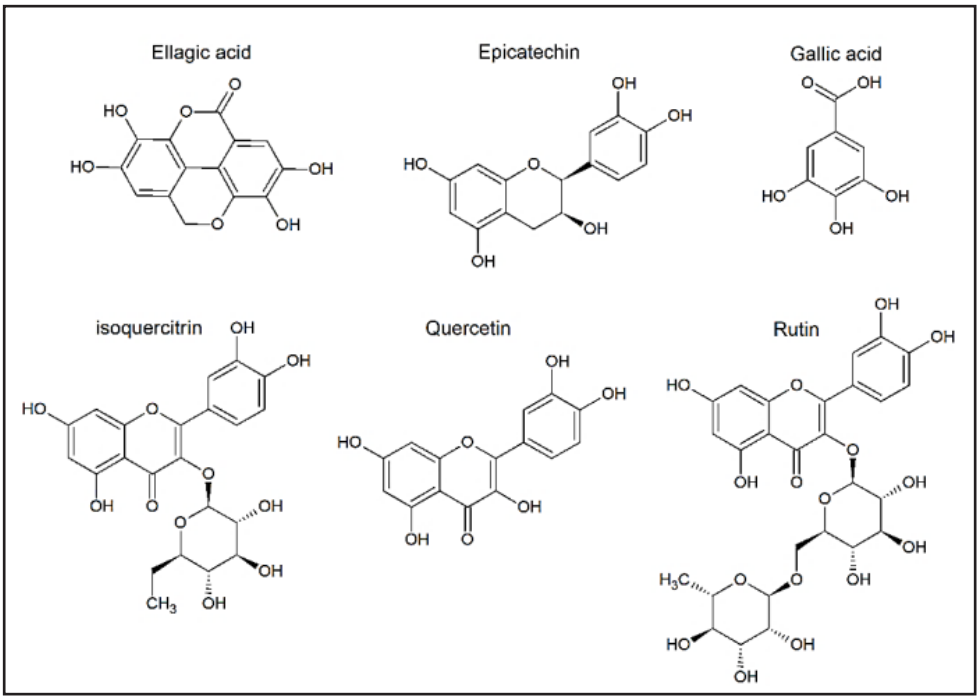
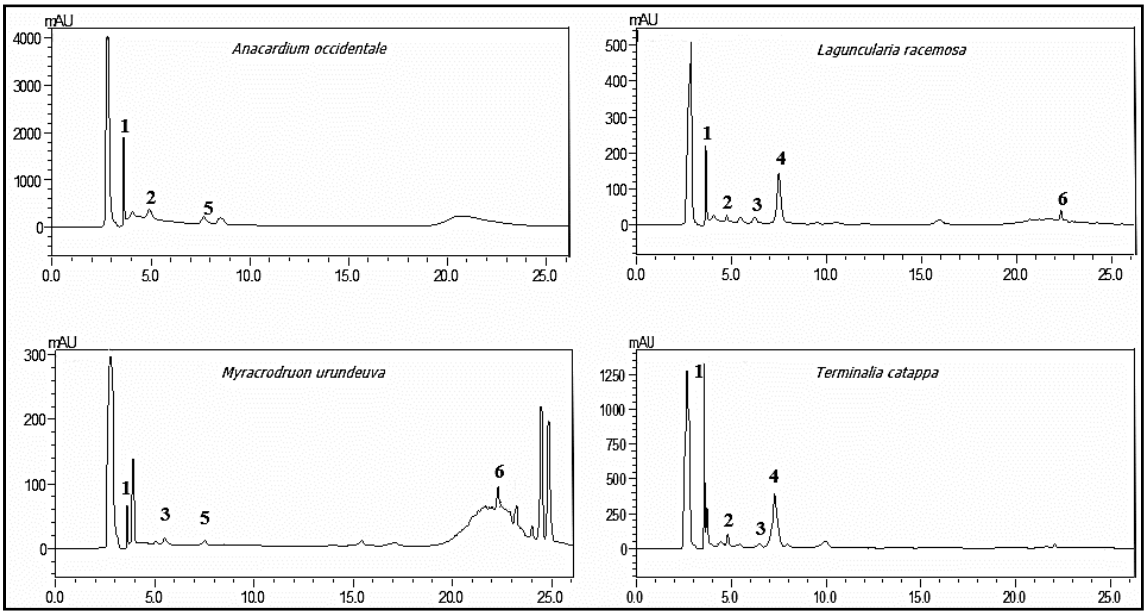


Figure 2 shows the HPLC chromatograms of the studied plant species, and Table 3 reports the quantifications of compounds.

Figure 2 – Characterization of phenolic compounds by HPLC-DAD (UV 350 nm)



1: Gallic acid; 2: Epicatechin; 3: Rutin; 4: Ellagic acid; 5: Isoquercitrin; 6: Quercetin

Table 3 – Quantification of phenolic compounds by HPLC-DAD (350 nm)

No.	T_R	Compounds	*A. occidentale*	*L. racemosa*	*M. urundeuva*	*T. catappa*
1	3.47	Gallic acid	12.07±0.09	1.24±0.08	0.16±0.02	4.78±0.04
2	4.82	Epicatechin	10.06±0.08	1.16±0.01	ND	2.48±0.03
3	6.42	Rutin	ND	1.14±0.02	0.70±0.02	1.34±0.01
4	7.12	Ellagic acid	ND	4.17±0.16	ND	14.85±0.15
5	7.52	Isoquercitrin	7.10±0.14	ND	1.02±0.03	ND
6	22.33	Quercetin	ND	0.46±0.01	1.34±0.02	ND

All tests were performed in triplicate, and the results are expressed as the mean ± standard deviation. Concentration: mg g^{-1} extract; T_R: retention time in minutes, ND: not detected.

Gallic acid was present in all extracts and displayed both antioxidant and pro-oxidant actions, depending on the reaction medium. Gallic acid and its derivatives have been gaining attention, not only because of their antiradical activity but also due to their potential to induce selective apoptosis. However, the pro-oxidant aspect of this molecule gives rise to the latter effect. The versatile action of anti- and pro-oxidant behavior generates diverse biological activities, including antitumor, antimicrobial, antimelanogenic and anticholesterol (Badhani et al., 2015). Gallic acid was active against three Candida strains, with MICs between 12.5 and 100.0 μg mL^{-1} (Li et al., 2017). Thus, its dual action about free radicals could favor the antifungal activity.

Epicatechin was identified in higher amounts in the *A. occidentale* extract and minor amounts in extracts of *L. racemosa* and *T. catappa*, respectively. Epicatechin is part of the catechins group, a subclass of flavonoids known as flavan-3-ols. It is abundant in green tea, black tea, cocoa, grapes, and fava beans. It is also found in lower concentrations in fruits such as raspberries, blackberries, apples, cherries, oranges and pears (Álvarez-Cilleros et al., 2018). It has several biological properties, such as antioxidant, antimicrobial, anti-inflammatory, antiviral, antidiabetic, anticancer and cardioprotective (Miranda-Buendia et al., 2022). It is believed that the anticancer and apoptosis-inducing properties of green tea are mediated by its polyphenolic constituents, particularly catechins. Several reports have shown that green tea

polyphenol (-)-epigallocatechin-3-gallate (EGCG) and epicatechin (EC) are among the most effective chemopreventive and apoptosis-inducing agents present in the beverage.

Rutin was identified in extracts of *L. racemosa*, *M. urundeuva* and *T. catappa*, with concentration varying from 0.70 at 3.11 mg g^{-1} of extract. *T. catappa* had the best result. Considered a potent nutraceutical molecule, rutin is a promising compound for the pharmaceutical and cosmetic industries, based on several reported pharmacological activities, such as antioxidant, anti-inflammatory, antihypertensive, anticancer, antidiabetic, cytoprotective, vasoprotective and cardioprotective actions (Semwal et al., 2021). Its antifungal activity showed MIC of 37.5 µg mL^{-1} against four strains of *C. albicans* (Ivanov et al., 2020). Rutin cannot only be considered purely as an antioxidant since, under certain reaction conditions, it can also display pro-oxidant activity. This unexpected behavior could explain, in part, the observed toxicity of some flavonoids *in vivo* (Kessler et al., 2003).

Ellagic acid was detected in the extract of the species *L. racemosa*, which showed a concentration of 4.17 mg g^{-1} of extract and in the extract of *T. catappa*, which showed a value substantially higher than the other compounds identified in the study, with a concentration of 14.85 mg g^{-1} of extract. Ellagic acid is a phenolic lactone derived from gallic acid, naturally occurring in medicinal plants and fruits such as strawberries, raspberries, grapes and pomegranates. It has antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antimutagenic, antiviral and anticancer properties (Ahad et al, 2014; Kumar et al., 2021). The antifungal activity of ellagic acid was investigated, and the results showed activity against the six strains tested (Rossatto et al., 2021).

Isoquercitrin was the main flavonoid identified in extracts of *A. occidentale,* and it is also present to a lesser extent in *M. urundeuva*. One of the main glycosidic forms of quercetin, isoquercitrin, is widely distributed in the plant kingdom and can be found in leaves, fruits and cereal grains, as well as in beverages such as wine and tea (Hasumura et al., 2004). It has antioxidant, anti-inflammatory, antidiabetic, anti-

allergic, cardioprotective and anticancer activity (Valentová et al., 2014). In the study carried out by Kim et al. (2019), isoquercitrin isolated from aerial parts of Aster yomena showed MIC of 2.5 μg mL^{-1} against the yeast *C. albicans*, in addition to showing good synergism with amphotericin B and fluconazole, increasing their antifungal potential.

Quercetin was identified in extracts of *L. racemosa* and *M. urundeuva*. It is a flavonoid of the flavonol subclass and is one of the most abundant *in nature*, found in practically all parts of plants, such as leaves, fruits, branches, stems and roots (Panche et al., 2016). It has several benefits to human health and excellent bioactivities such as antioxidant, anti-inflammatory, antimicrobial, antiviral and anticancer (Hai et al., 2020; Sun et al., 2020; Wang et al., 2020). It had strong antifungal activity against *C. albicans* strains isolated from gynecological patients in Poland, where the compound presented an average MIC of 64 μg mL^{-1} (Janeczko et al., 2022).

3.2 Determination of antifungal activity

Infections caused by fungi of the genus *Candida* are opportunistic. They can either be localized or systemic in humans, varying from superficial infections of the skin or mucous membranes to generalized infections. These infections are caused by approximately 200 different species of yeasts that normally live in diverse body niches (Spampinato and Leonardi, 2013). *C. albicans* is the main etiological agent of candidiasis and accounts for about 60% of the species of the genus isolated in clinical samples (Silva et al., 2021). Studies have shown that flavonols and chalcones have the potential to damage the fungal cell membrane and that tannins have good synergy with antibiotics (Ekambaram et al., 2016; Seleem et al., 2017). Table 4 shows the results of antifungal activity against *C. albicans* yeast strains.

The classification of the antifungal efficiency of the extracts used in the assay was done according to the MIC values, following the indications of Sartoratto et al. (2004), who considered MICs lower than 500.0 μg mL^{-1} as strong activity; MICs between 500.0-1500.0 μg mL^{-1} as moderate activity, and MICs above 1500.0 μg mL^{-1} as

low activity. Based on this scale, *A. occidentale* and *L. racemosa* presented strong activity, with MICs varying from 19.0 to 310.0 µg mL^{-1}.

The species *A. occidentale* showed the highest antifungal activity against the four strains tested, with MICs ranging from 19.0 to 78.0 µg mL^{-1}. These findings support the use of the species' bark in traditional medicine to treat mycoses. Andrade Júnior et al. (2018) demonstrated that *A. occidentale* has other organs besides the bark, such as leaves and flowers, with potential against fungi of the genus Candida. Observing the phenolic composition of *A. occidentale* bark ethanol extract (Table 4), it presents three compounds: gallic acid, epicatechin and isoquercitrin, which are to a lesser extent, in the other species.

Table 4 – Minimum inhibitory concentration (µg mL^{-1}) of selected plant extracts

		Candida albicans		
	Species	**Candida albicans**	**0104**	**0105**
	ATCC	0102	0104	0105
A. occidentale	39.0	78.0	78.0	19.0
M. urundeuva	150.0	620	78.0	78.0
L. racemosa	78.0	150.0	78.0	78.0
T. catappa	39.0	310.0	620.0	620.0
Fluconazole	0.5	0.25	0.5	0.5

3.3 Molecular docking - evaluation of in silico results

The molecular docking simulations were performed to understand the possible action mechanism of the main molecules of extracts analyzed in the *Candida albicans* strains. All the compounds analyzed, except for gallic acid, exhibited higher interaction energy (ΔG) values with Sap5 protein compared to the standard value (ΔG -6.0 kcal mol^{-1}) (Hanwell et al., 2012). Concerning the RMSD results, only fluconazole showed RMSD values outside the ideal parameter of 2.814 Å. The inhibition constants (Ki) and pKi ranges observed were from 1.28 x 10^{-7} to 4.17 x 10^{-7} and 6.38 to 6.89, respectively. Table 5 shows the affinity energy, RMSD, Ki and pKi values of the complexes formed.

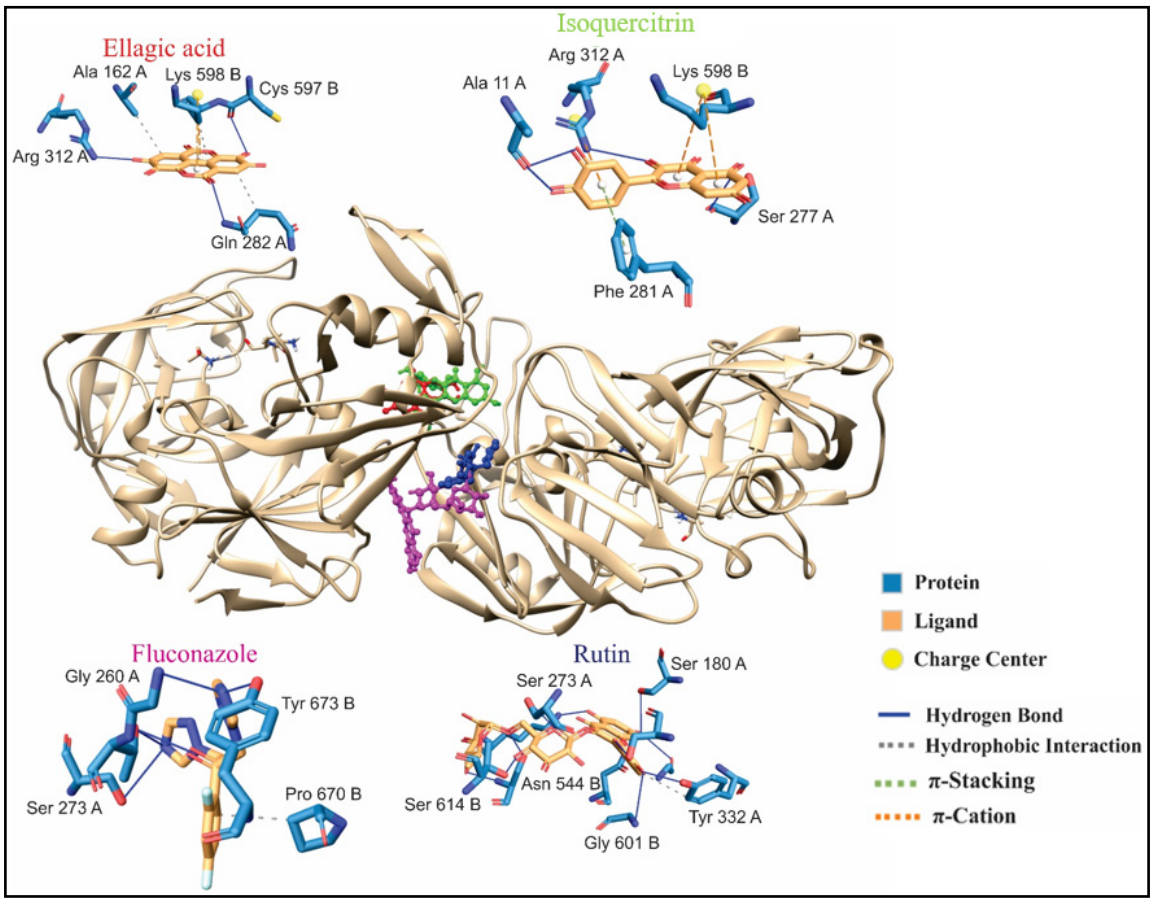
Table 4 – Affinity energy, Ki, pKi and RMSD of the complexes formed with *Candida albicans*

Receiver	Ligands	Energy (kcal moL^{-1})	Ki	pKi	RMSD (Å)
Candida albicans Sap5	Ellagic acid	-9.4	1.28×10^{-7}	6.89	0.057
	Epicatechin	-8.7	4.17×10^{-7}	6.38	1.428
	Gallic acid	-5.9	4.71×10^{-5}	4.33	0.290
	Isoquercitrin	-9.3	1.51×10^{-7}	6.82	1.650
	Quercetin	-8.9	2.97×10^{-7}	6.53	1.696
	Rutin	-9.0	2.51×10^{-7}	6.60	1.907
	Fluconazole	-6.9	8.70×10^{-6}	5.06	2.814

Therefore, all compounds analyzed are high affinity energy ligands with the Sap5 protein ($\Delta G \leq -6$), except for the gallic acid molecule. Among them, ellagic acid, isoquercitrin and rutin registered the best capacity to inhibit the enzymatic activity of the Sap5 protein. Then, the plant species with better antifungal activities include ellagic acid, isoquercitrin and rutin within their constituents, which already have demonstrated activities against Candida species (Kim et al., 2019; Rossatto et al., 2021).

There are many important enzymes for Candida species surveillance, and Sap5 was the one chosen to compare, by computational studies, the antifungal activity of the main constituents detected in the extracts by the HPLC analysis. Among the three main compounds pointed out in the *in silico* studies, *A. occidentale* extract only presented isoquercitrin, which is not detected in the other plant extracts then *A. occidentale* activity should probably be due to the higher content of isoquercitrin and the synergism of the several compounds present in the extract.

Figure 2 – Binding sites between the *Candida albicans* Sap5 and ellagic acid (red), isoquercitrin (green) and rutin (blue) ligands



The parameters used in molecular docking simulations (grid box centers, sizes, spacing and completeness) are shown in Table 5.

Table 5 – Parameters used in the molecular docking simulations: grid box centers, sizes, spacing and exhaustiveness

Protein	Center			Size			Spacing	Exhaustiveness
Sap5	X	Y	Z	X	Y	Z		
	17.972	22.333	41.583	84	92	126	0.753	64

The interactions and distances of the complexes of Sap5 ligands with the most negative values of ΔG (which makes the interaction thermodynamically more favorable)

will be discussed here. This ligand also registered only strong hydrogen bonds with the Gln 282 A (2.08 Å), Arg 312 A (2.33 Å), and Cys 597 B (2.75 Å) residues, and π-cation interactions with Lys 598 B (5.62 Å and 5.41 Å), respectively. The Sap5-epicatechin complex exhibited three hydrophobic interactions and two strong hydrogen bonds with the Gln 282 A (3.85 Å), Lys 598 B (3.75 Å), Gln 623 B (3.69 Å) and Glu 278 A (1.97 Å) and Tyr 284 (2.20 Å) residues. On the other hand, two strong and one moderate hydrogen bond were registered for the isoquercitrin molecule, with the Ala 11 A (2.30 Å and 2.15 Å), Arg 312 A (2.78 Å) and Ser 277 A (3.49 Å) residues, respectively. Furthermore, this ligand showed two π-cation interactions, with Arg 312 A (5.88 Å) and Lys 598 B (5.58 Å), and one π-stacking interaction with Phe 281 A (5.21 Å). The Sap5-rutin complex exhibited 12 strong and two moderate hydrogen bonds with Ser 180 A (3.07 Å), Thr 261 A (2.22 Å and 2.23 Å), Ser 313 A (1.93 Å), Tyr 332 A (2.13 Å), Asn 544 B (2.28 Å), Gly 547 B (2.89 Å), Lys 612 B (2.34 Å, 1.82 Å, 2.37 Å), Ser 614 B (2.11 Å and 2.03 Å), Ser 273 A (3.24 Å) and Gly 601 B (3.35 Å), respectively. Furthermore, only one hydrophobic interaction was registered between rutin and Tyr 332 A (3.86 Å) of the Sap5 protein. The quercetin ligand interacted through π-stacking and π-cation with Phe 281 A (5.21 Å), Arg 312 A (5.88 Å), and Lys 598 B (5.58 Å), respectively. This ligand also registered three strong and one moderate hydrogen bond with Ala 11 A (2.30 Å and 2. 15 Å), Arg 312 A (2.78 Å) and Ser 277 A (3.49 Å), respectively.

4 CONCLUSIONS

The extracts of *Anacardium occidentale, Myracrodruon urundeuva, Laguncularia racemosa* and *Terminalia catappa* showed a remarkable ability to effectively inhibit *Candida albicans* yeast strains, exhibiting a minimum inhibitory concentration of less than 500.0 μg mL^{-1}. These results indicate the therapeutic potential of these four species in antifungal treatments against *Candida albicans*. The chemical compounds identified in the extracts, such as ellagic acid, isoquercitrin and rutin, played a crucial

role in this action. *In silico* analyses, these compounds showed significantly strong affinity energies with the Sap5 protein of *C. albicans*, surpassing even the reference drug (fluconazole). It is important to note that the mode of action of the phenolic compounds differs between them. While rutin acts on the same site as fluconazole, ellagic acid and isoquercitrin act on protein allosteric sites. This suggests that they could be used synergistically with fluconazole in the treatment of infections caused by strains of *C. albicans*. These results encourage further studies with these plants, aimed at isolating the substances present in the extracts, which could contribute to developing antifungal activities and possible pharmaceutical applications in the future.

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