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Phenological phases and plant tissues of *Commiphora leptophloeos* influence the antibiofilm activity of *Candida spp.*

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Abstract

Background

The plant metabolites production is affected by environmental and biological factors, especially in semiarid environments. Thus, its phonological phase may be an indicator of its phytochemical composition, although studies with this approach are scarce. The objective of this work was to investigate whether changes in the phonological phase of *Commiphora leptophloeos* influence its antibiofilm potential of *Candida* spp.

Methods

We tested ethanol and hexane extracts from leaves and barks of the plant, in two phonological phases, i.e. with leaves and flowers (harvest 1) and with fruits (harvest 2), were tested against three stains: *Candida albicans* URM 4387, *C. krusei* URM 6452 and *C. tropicalis* URM 4262. The effect of the extracts was evaluated in planktonic cells for determination of the Minimum Inhibitory Concentration and the Minimum Fungicidal Concentration using the microdilution technique. We evaluated the extracts effect in biofilm formation and in pre-formed biofilms by colorimetric technique with crystal violet and the cell viability technique with methylene blue.

Results

All extracts showed inhibitory effect between the concentrations of 2mg/ml and 1mg/ml, as well as fungicidal effect in concentrations equal or higher than 2mg/ml. All the extracts were able to decrease viable cells in the biofilms by more than 50%. There was no synergism between the extracts and Fluconazole. The extracts of the second harvest (fruiting season) showed better results in the activity on the biofilm formation of *Candida* spp. The extracts of leaf from the first harvest showed higher inhibitory capacity against the pre-formed biofilms, and among the bark extracts, the second harvest was more efficient. The high performance liquid chromatography (HPLC) showed that there was a greater amount of Gallic acid in the bark of *C. leptophloeos* than in the leaves, with no difference between the harvests for this part of the plant. As for the leaves, the amount of Gallic acid found in the second harvest was higher than that quantified in the first harvest.

Conclusions

The promising capacity of *C. leptophloeos* extracts against *Candida* spp. biofilms is perceived. Furthermore, the results imply that the phenological phases are not the only factor to exert influence on the antibiofilm effect; the part of the plant used should also be taken into consideration.

Background

Yeasts of the genus *Candida* are responsible for several infections annually (KUMAR et al., 2020). Moreover, these yeasts have become increasingly resistant to known antifungal drugs, especially azoles, the greater class of antifungal medicine, against *Candida* (PEREIRA et al., 2020). Alternatively, plant secondary metabolites (MS) have shown as a promising source of molecules with antifungal properties (KUMAR et al., 2020), which usually have low molecular weight and possess biological activities (PEREIRA; CARDOSO, 2012). Nevertheless, the

phenological phase of the plant can interfere with the chemical composition of these MS (CHEN et al., 2012; ÇIRAK et al., 2007; INÁCIO et al., 2016). However, this is a theme still little explored.

Biological activity is influenced by plant age, temperature, seasonality, and phenological phases (GOBBO-NETO; LOPES, 2007). The season of plant harvest is the most influential factor since the amount and nature of active compounds are not constant throughout the year (CHEN et al., 2012; GOBBO-NETO; LOPES, 2007). The variations in the composition of SM depend on each species and tissue of the plant (LI et al., 2020).

Among the biological activities coming from these MS is the antifungal action, which can be exerted by the plant extract as a whole, by compounds isolated from the plant, or in conjunction with drugs already known and used, such as Fluconazole (GIORDANI et al., 2020). An example of this synergistic capacity with these drugs is the ethanolic extract of the *Commiphora molmol* bark resin that showed synergistic activity with ofloxacin and fluconazole, evidencing the potential of this plant (ARORA et al., 2021).

Commiphora leptophloeos is a medicinal plant known as Imburana-de-Cambão, widely distributed in the Brazilian semiarid region (SILVA et al., 2017). The bark extracts of *C. leptophloeos* showed antibiofilm activity of *Candida* spp. (DANTAS-MEDEIROS et al., 2021a). The leaf extracts showed moderate antifungal activity against *Candida krusei, C. parapsilosis*, and *C. glabrata* in planktonic cells (PESSOA et al., 2021). Thus, this species seems quite promising for evaluating the influence of its phenological phases on antifungal potential.

The main compounds identified in the leaves of *Commiphora leptophloeos* are orientin, iso-orientin, vitexin, isovitexin, isoquercitrin, and quercetin (DANTAS-MEDEIROS et al., 2021b). The peels also have gallic acid in their composition (PEREIRA et al., 2017), which has anti-inflammatory, antioxidant, antifungal, and antimicrobial properties (LIBERATO et al., 2022), besides phenolic acids and tannins, the latter can reduce *Pseudomonas aeruginosa* biofilms (TRENTIN et al., 2013).

In this context, this work tested the hypothesis that there is an alteration in the phytochemical composition of *Commiphora leptophloeos* leaves and bark as a result of the harvest season, and this variation influences its antifungal action. The effect of the extracts isolated and in association with the drug Fluconazole against biofilms of *Candida* spp. were tested.

Methods

Study area and collection of botanical material

The leaves and barks of five individuals of *Commiphora leptophloeos* were harvested at Buenos Aires property (07° 57' 45.6"'S; 38° 23' 02.3" W), a private area with well-preserved native vegetation, located in the municipality of Serra Talhada, Pernambuco, Brazil. The region has a hot semi-arid climate, type Bwh by Köppen classification (MEDEIROS et al., 2020), with a mean annual precipitation of 574 mm (SOUZA et al., 2016).

Commiphora leptophloeos is a deciduous species, with phenology influenced by photoperiod and by precipitation, given that it stores large water reserves (DE LIMA et al., 2012). This species starts losing its leaves in the rainy season (from the middle to the end of the season) and starts sprouting new leaves at the end of the dry season (DE LIMA et al., 2012). In the period from November to January (Table 1), in the transition between the dry and rainy seasons, the sprouting and beginning of flowering occur (LEITE; MACHADO, 2010), and between March and June, the rainy season, fruiting occurs (SILVA et al., 2020).

To evaluate how the phenological phase of *Commiphora leptophloeos* influences the antibiofilm activity, we collected in the morning of December 2020, during the leaves and flowering production stage, and in March 2021, during the fruiting season (Fig. 1).

Botanical material from five individuals was collected under the same environmental conditions, within an area of approximately 0.1 ha. The bark (~ 1kg of fresh material) was collected by removing it from the second or third branch, never exceeding 1/3 of its total circumference. Freshly expanded and healthy leaves (~ 800g of fresh material) were collected, noting if fungus or marks were absent of herbivore attacks (FILIZOLA; SAMPAIO, 2015). Leaves and bark were transported to the laboratory protected from light and immediately placed in a 40°C air circulation oven for five days for complete drying (DA SILVA et al., 2019).

Obtaining The Plant Extracts

Extraction was performed by macerating the leaves and barks separately using 99.5% ethanol (P.A.) for 72 hours, followed by filtration on Whatman paper (DA SILVA et al., 2019); the solvent was evaporated using an IKA RV 05 Basic rotary evaporator, never exceeding 45°C. The process was repeated, replacing ethanol with hexane (DA SILVA et al., 2019). The ratio used in the extraction was 1g of material to 5 ml of solvent. We obtained six extracts, two ethanolic extracts of the leaves from the two collections (EEL1, EEL2); two hexanoic extracts of the leaves from the two collections (HEL1, HEL2), and two ethanolic extracts of the barks from the two collections (EEB1, EEB2). The hexanoic bark extracts were unused since the amount obtained was low and thin to perform the analyses.

Determination Of Gallic Acid By High Performance Liquid Chromatography (HPLC):

Gallic acid, which is a phenolic compound with antifungal and antibiofilm activity for *Candida* spp. (GIORDANI et al., 2020), was identified and quantified in the ethanolic extracts using a Thermo Scientific Ultimate 3000 HPLC controlled by Chromeleon Chromatography Management System software, with a C18 column (250 mm × 4.6 mm; 5 µm).

The isocratic method was used, whereby the mobile phase consisted of acidified water, 2% acetic acid (phase A), and pure methanol (phase B), at a flow rate of 0.8 mL/min, wavelength ( ) of 270 nm, injection volume of 20 µl, and retention time of 10 min. The standard used in the standard curve was gallic acid at concentrations of 0.05, 0.10, 0.15, 0.20, and 0.25mg/ml. Other compounds were not tested due to the lack of standards in the laboratory for comparison. Gallic acid was identified by comparing its retention time with the corresponding standard (PEREIRA et al., 2017).

Strains And Determination Of Minimum Inhibitory Concentration (MIC) And Minimum Fungicidal Concentration (MFC):

Candida albicans (URM 4387), *C. tropicalis* (URM 4262), and *C. krusei* (URM 6352) strains were obtained through collaboration with the Department of Biology of the Universidade Federal do Cariri (UFCA), Brejo Santo, Ceará,

grown at 37 ºC and maintained on Agar Yeast Extract Peptone Dextrose (A-YEPD: HIMEDIA) medium under refrigeration.

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent responsible for inhibiting the growth of a microorganism (bacteria or fungus), i.e., has bacteriostatic or fungistatic activity (ANDREWS, 2001). The minimum fungicidal concentration (MFC) is the lowest concentration of the therapeutic agent capable of preventing 100% microbial growth (BHATTI et al., 2014).

To determine the MIC, in 96-well plates, 100 µl of C-YPD, 50 µl of each concentration of the extract (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL and 0.125 mg/mL) and 50 µl of the yeast cell suspension (106 cells/ml) were added in each well (DANTAS-MEDEIROS et al., 2021a). The plates were incubated at 37°C for 24 hours under agitation (60 rpm). The MIC was considered the minimum concentration necessary to inhibit biofilm formation (turbidity absence at the bottom of the well) compared to the negative control (no extract added).

The MFC was determined using the drop technique, in which 10 µl of the contents of the MIC, as well as 10 µl of the contents of the two concentrations of the extracts higher and lower than the MIC value, were seeded onto Petri plates containing YEPD Agar (JOVITO, 2016), and incubated at 37°C for 24 hours. The MFC was determined by the lowest concentration at which there was no growth of yeast colonies on the surface of the YEPD Agar.

For both analyses, the positive control was composed of the addition in the wells of C-YEPD plus the yeast cell suspension; the negative control was composed of the addition of C-YEPD plus the plant extracts. All experiments were performed in triplicate.

Determination Of Biofilm Formation Capacity

The capacity of biofilm formation by the selected strains of *Candida* spp. was evaluated according to the total biomass of biofilms following the methodology described by Silva et al. (2009). The yeasts were previously cultivated in A-YEPD for 18 h at 37 ºC. After this period, the yeast cells were suspended in Sterilized Distilled Water (SDE) and the cells number was adjusted to 106 cells/mL with the help of a McFarland scale. From this yeast cell suspension, 50 µl were transferred to 96-well flat-bottom plates containing Yeast Extract Peptone Dextrose Broth (C-YPD) and incubated under stirring at 60 rpm and 37 oC for 24 h. After this period, the supernatant from the wells was pipetted and discarded (SILVA et al., 2009).

The biofilm formation was evaluated by the colorimetric method with crystal violet, i.e., after biofilm growth and removal of the supernatant, 100 µl of a 1% crystal violet solution was added to each well for 5 min. After this period, the crystal violet was removed with the help of a pipette followed by the addition of 100µl of Absolute Ethanol (99.4 P.A.). After solubilization and homogenization of the violet crystal, the entire volume of each well was transferred to a new 96-well plate and subjected to Optical Density (OD) reading (ELISA MULTISCAN GO, Thermo Scientific) at a wavelength of 492 nm (TAFF et al., 2013).

Biofilm-forming yeast was considered those with OD readings greater than 0.1, while for biofilm formation intensity the yeasts were classified as strong (> 0.3), moderate (> 0.2 and < 0.3), and weak (> 0.1 and < 0.2), according to Pfaller et al. (2006). The negative control was performed using only C-YPD. The entire experiment was performed in triplicate.

Evaluation Of The Anti-biofilm Effect Of Extracts By Microdilution Technique: On Biofilm Formation

To evaluate the effect of the extracts on biofilm formation, 100 µl of C-YEPD, 50 µl of each extract concentration (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL and 0.125 mg/mL) and 50 µl of the yeast cell suspension (106 cells/ml) were used in 96-well plates, which were incubated at 37°C for 24 hours under agitation (60 rpm). The plates also contained wells without the extracts, for growth control. For quantification of the biofilm biomass, the colorimetric method with crystal violet was used and read in Thermo Scientific GO Multiscan ELISA plate reader with a wavelength of 492nm. This method is based on the crystal violet's ability has in a bind to negatively charged molecules present in the yeast cell wall, as well as in the extracellular polysaccharide matrix produced in biofilms, so there is a direct correlation between the amount of biofilm (biomass) formed and the OD value obtained (NEGRI et al., 2010).

In Preformed Biofilms:

To form the biofilms, 100 µl of C-YEPD and 50 µl of the yeast cell suspension (106 cells/ml) were added to 96-well plates and incubated at 37°C for 24 hours under stirring (60 rpm). After this period, to check the influence of the extracts on already formed biofilms, 50 µl of each extract concentration (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, and 0.125 mg/mL) were added to each well, leaving the extracts in contact with the biofilms, under agitation (60 rpm), for 24 hours. All supernatant was removed, and the biofilm biomass was quantified by the colorimetric method with crystal violet, as described in the previous item.

Evaluation Of The Antibiofilm Effect Of The Extracts By Methylene Blue Staining

This method allows us to estimate the percentage of viable cells present in a sample. It is based on the fact that living cells contain enzymes capable of reducing methylene blue to colorless compounds. When cells are immersed in methylene blue, which enters the interior of the cell, the enzymes in the living cells decolorize the coloring matter. Dead cells, on the other hand, in which the enzyme is inactive, do not react remaining stained blue (KWOLEK-MIREK; ZADRAG-TECZA, 2014).

Thus, this step was performed to evaluate, through an independent cultivation technique, the effect of the extracts on the cell viability of the yeasts studied, both in biofilm formation and pre-formed biofilms, as previously described. We followed the methodology described by Ceccato-Antonini, Tosta, and Silva (2004) with modifications.

After contact with the different concentrations of the extracts, both in biofilm formation and in preformed biofilms, the supernatant was removed and added to each well of the multiwell plate 200 µl of PBS buffer and, with the help of a pipette, the cells were gently detached from the bottom of the well, transferred to an Eppendorf tube and centrifuged for 5 min at 10,000 rpm. The supernatant was discarded and again 200 µl of PBS buffer was added. This washing process was performed 3 times to remove the extract or any other dirt, avoiding interference with the reading of the results. Each sample was then added 200 µl of methylene blue dye (1%) and left in contact with the yeast cells for 10 min. After this period, using a Neubauer chamber and clear field optical microscope, a total of

100 cells per sample were then observed and the estimated viable cells were calculated (% viability = (number of unstained cells/number of total cells) x 100).

Effect of *C. leptophloeos* extracts on yeast cell morphology:

To assess whether the extracts influenced cell structure and pseudo hyphae formation, slides of the yeasts exposed to the extract concentrations of 1mg/ml and 0.125mg/ml were prepared. In this step, it was observed under microscopy if there was a change in the yeast cell morphology, if they emit or not germ tube, if there were or not asexual reproduction cells (buds), or if the yeasts formed or not pseudo hyphae, parameters related to virulence and biofilm formation capacity of the yeasts (CIUREA et al., 2020; MCKENZIE et al., 2010).

Statistical Analysis

The data obtained for each sample concentration were checked for normal distribution and then analyzed by one-way ANOVA and subsequently by Tukey's test using the R software. $p < 0.05$ was considered significant.

Results

In this study it was evaluated whether the phenological phase that the plant is in at the time of collection influences the antibiofilm capacity of *Candida* spp. It was noticed that all extracts of the leaves and bark showed inhibitory effect on both biofilm formation and pre-formed biofilms. Furthermore, it was noticed that with regard to biofilm formation, the extracts from the second collection showed greater inhibitory effect than those from the first collection. In the case of pre-formed biofilms, the leaf extracts from the first collection were better than those from the second, and the bark extract from the second collection was more effective than the first. The presence of gallic acid was also verified in the barks and leaves of *C. leptophloeos*, and the barks contained a significantly higher amount than the leaves, although with no difference between the collections. In the leaves, the difference in the amount of gallic acid is noticeable, since in the fruiting season (second harvest) the leaves contained more gallic acid than when in the vegetative season (first harvest).

Determination Of The Minimum Inhibitory Concentration (MIC) And Minimum Fungicidal Concentration (MFC)

The MIC of the extracts ranged from 1 mg/ml to 2 mg/ml (Table 1). The lowest MIC values obtained were 1.75 mg/ml for extracts EHF1 and EEC1 against *C. albicans* URM 4387, 1 mg/ml for extract EEF2, against *C. tropicalis* URM 4262 and 1.5 mg/ml for all extracts against *C. kruzei* URM 6352.

All extracts (from both harvests) showed fungicidal effect on the strains studied, with concentration equal to or greater than 2 mg/ml (Table 2).

Biofilm Forming Capacity

All strains were previously tested for their biofilm forming ability, and classified as strong (OD > 0.3), moderate (OD > 0.2 < 0.3) or weak (OD > 0.1 < 0.2). In this regard, *C. albicans* URM 4387 (OD = 0.35) and *C. krusei* URM 6352

(OD = 0.31) were considered as strong biofilm formers, while *C. tropicalis* URM 4262 (OD = 0.29) was considered as moderate biofilm formers.

Effect Of Extracts On Biofilm Formation

The effect of ethanolic and hexanolic extracts of *C. leptophloeos* leaves and bark on *Candida* spp. biofilm formation was observed through quantification of biofilm biomass, which was determined by colorimetric technique with crystal violet and subsequent reading of the respective Optical Densities (ODs).

The OD values indicate the amount of biomass of fungal biofilms formed in each well of the microdilution plate. Thus, it is considered that the lower the OD reading, the lower the biomass present, consequently, the better the action of the extract in inhibiting biofilm formation.

The effect of the extract EEL1 on the *C. krusei* strain was significant (Fig. 2a). Although the OD value of the EEL1 extract was higher than that of the control, there is an indirect relationship between the concentrations of the extract and the observed biomass. However, it is not possible to see this pattern when analyzing the effect of EEL1 extract on *C. albicans* and *C. tropicalis* strains (Fig. 2a). For these yeasts, the opposite happens: in the higher concentrations of the extracts the OD values are higher when compared to the lower concentrations of the extracts. Thus, one of the possible explanations for the results obtained by this technique is that the effect of the extract was not inhibitory, but favored the growth of the biofilm.

The extract HEL1 was also efficient when tested against *C. krusei* URM 6352 (Fig. 2b). This time, the OD value of all concentrations tested were lower than the control, showing the inhibitory effect of this extract. Thus, we highlight that the extract HEL1 was the only one that, when compared to the control, inhibited the development of biofilms by *C. kruzei* URM 6352.

When tested against *C. albicans* URM 4387 and *C. tropicalis* URM 4262, the extract HEL1 showed a higher reading than the control, contrary to what was expected, that is, also causing a possible favoring of biofilm growth by these yeasts.

The EEB1 extract showed higher OD value than all controls of the tested yeasts (Fig. 2c), besides there was no inverse relationship between the concentration of the extract and the amount of biomass of the biofilms.

The ethanolic extract of leaves from the second collection (EEL2) was able to inhibit the biofilm development of *C. krusei* and *C. tropicalis* strains (Fig. 3a), as the OD values were lower than those of the controls.

The hexanic extract of leaves from the second collection (HEL2) (Fig. 3b) was efficient against the formation of biofilms of *C. albicans*, in this case it is noticed that besides the OD values being lower than the control, the readings are indirectly related to the concentration, that is, the higher the concentration of the extract, the lower the amount of biofilm biomass for this yeast. For the other two *Candida* strains, this relationship is not observed.

The efficiency of the ethanolic extract of bark from harvest 2 (EEB2) (Fig. 3c) is perceived on the formation of biofilms of *C. albicans* URM 4387, which in the same way as HEL2, showed lower OD values than the control, and with the ratio OD value/concentration of the extract met.

Effect Of Extracts On Preformed Biofilms

For this analysis, the ODs of the preformed biofilms without action of the extracts were read, i.e., the positive controls and are used as a comparative standard to analyze the effects of the extracts on preformed biofilms of the studied yeasts.

It was observed that the extract EEL1 (Fig. 4a) presented effect against the preformed biofilms of *C. tropicalis* URM 4262, because, except for the concentration of 2 mg/ml, the OD readings were lower in the presence of the extracts, when compared to the positive control. For this same extract, the OD values of *C. albicans* and *C. kruzei* biofilms were all higher than the positive control, and here there may have been a positive influence of this extract on biofilm development.

The OD values after action of the HEL1 extract (Fig. 4b) were lower than all the controls of the tested strains. However, the expected correlation was not presented, that is, the more concentrated the extract, the lower the biofilm biomass.

Extract EEB1 (Fig. 4c) also showed lower OD values than controls for *C. albicans* and *C. tropicalis* strains, again without the expected pattern. This pattern, however, is observed when the extract is in contact with the biofilm of *C. krusei*, whose reading increases as the concentration decreases, even though the OD is higher than that of the control.

The EEL2 extract (Fig. 5a) showed a significant effect against the pre-formed biofilms of *C. albicans* URM 4387 and *C. tropicalis* URM 4262, however we see that the highest concentration had a higher OD reading than the other concentrations, again suggesting that the extracts favored the development of the biofilms.

The extract HEL2 (Fig. 5b) showed antibiofilm effect against the *C. krusei* strain URM 6352. Here we notice the opposite effect of extract EEL2 (Fig. 5a), because against this yeast the extract showed OD value lower than the control, especially at the highest concentration (2 mg/ml) showing its ability to decrease the biofilm biomass. In EEB2 extract (Fig. 5c), the OD values were lower than all controls for the three yeasts studied, indicating the inhibitory effect of this extract.

Morphological Analysis Of The Yeasts

It was observed that in the presence of the extracts (EEL1, HEL1, EEB1, EEL2, HEL2 and EEB2), the yeasts did not multiply, thus showing a smaller number of cells, visually, when compared to the positive control (cells without the extract). This control showed a large amount of dividing cells (budding), while the cells of the yeasts that were subjected to the action of the extracts showed a much smaller amount of buds and pseudo hyphae (Fig. 6).

Cellular Viability - Methylene Blue

This methodology allows the observation of the quantity of non-viable cells, through methylene blue staining and cell count. Thus, it is considered that the greater the number of non-viable cells, the greater the antifungal and antibiofilm capacity of the extract.

On Biofilm Formation

All extracts, from both harvests, were able to decrease the formation of biofilms of *Candida* spp. strains (Tables 3, 4 and 5). In extract EEL1, its strong staining at the highest concentration (2mg/ml) prevented the visualization of cells, so it was not possible to count the cells affected by this extract at this concentration. However, it can be seen that the 1mg/ml concentration was able to decrease more than 50% of the cells of all the yeasts studied, having the greatest effect on *C. tropicalis* URM 4262, decreasing more than 86% of the cells (Table 5).

The extract HEL1, at the concentration of 2mg/ml decreased 84.6% of the cells of *C. albicans* URM 4387 (Table 3), 94.6% of the cells of *C. krusei* URM 6352 (Table 4) and 87.6% of *C. tropicalis* URM 4262 (Table 5). When the effects of ethanolic (EEB1) and hexanic extract of leaves (HEL1) on yeasts were compared, the HEL1 extract had a greater effect at all concentrations (Tables 3, 4 and 5). The EEB1 extract was also more efficient against *C. krusei* URM 6352 yeasts (Table 4), making 96.3% of the cells inviable. In addition, the bark extract from the first collection showed greater effect than the leaf extract (EEL1) on the tested yeasts (Tables 3, 4 and 5).

Extract EEL2 was most efficient against *C. tropicalis* URM 4262, decreasing more than 89% of the cells at the 1mg/ml concentration (Table 5). Extract HEL2 was most efficient at the 2mg/ml concentration against all *Candida* strains (Tables 3, 4 and 5). When comparing the effects of the two extracts on yeast, extract HEL2 showed better results in all concentrations.

Similarly, the EEB2 extract also showed greater efficiency at the highest concentration (2mg/ml) against *C. krusei* URM 6352 (Table 5) and *C. tropicalis* URM 4262 (Table 5), but against *C. albicans* URM 4387 the concentration of 0.5mg/ml was more efficient, as there was no statistically significant difference compared to the higher concentrations. When compared to the leaf extract, the EEB2 extract was more efficient in inhibiting biofilm formation.

When comparing the differences in the effects of the extracts from different collections, it is noticed that even at the lowest concentration, i.e. 0.125mg/ml, the extract EEB2 had a greater effect than EEB1. Among the leaf extracts, the inhibitory capacity of the ethanolic extract of the second collection (EEL2) was higher than that of the first (EEL1). However, in the hexanic extracts, the opposite occurs; the extract EHF1 has a greater effect on yeasts than HEL2.

This implies that the phenological phase of the plant is not the only factor that influences the potentiality of the extracts. Although all had some effect on *Candida* spp. it is necessary to stick to the part of the plant and the solvent used.

On Preformed Biofilms

When tested against the pre-formed biofilm of *C. albicans* URM 4387 (Table 6), extract EEL1 was able to decrease the biofilm cells by 52% at the 1mg/ml concentration. Again, it was not possible to observe the amount of viable cells at the 2 mg/ml concentration due to the staining of the extract. However, at the 1 mg/ml concentration the efficiency of the extract is noticeable, as it was able to decrease more than 70% the viable cells of *C. krusei* URM 6352 (Table 7) and *C. tropicalis* URM 4263 (Table 8) biofilms.

Extract HEL1 (Table 6) was more efficient against *C. albicans* URM 4387 biofilm decreasing viable biofilm cells by more than 70% at the concentration of 1 mg/ml. When the comparison between the leaf extracts, EEL1 and HEL1 was made, it was noticed that on pre-formed biofilms the ethanolic extract had a greater inhibitory effect. The EEB1 extract was able to reduce more than 80% of the *C. albicans* biofilm (Table 6) and *C. tropicalis* (Table 8), and when compared with the effect of the leaf extract, EEB1 showed better effect in all concentrations.

The EEL2 extract was more efficient against *C. albicans* (Table 6) and *C. krusei* (Table 7) biofilms with more than 70% reduction of these biofilms at the concentration of 1 mg/ml. The biofilm of *C. albicans* URM 4387 suffered the greatest reduction (67% of biofilm) in contact with the extract HEL2 (Table 7) when compared to the other yeasts. As in the first collection, HEL2 was more efficient than HEL2 when tested against preformed biofilms. In contact with the EEB2 extract, *C. krusei* (Table 7) had a greater reduction, since the extract turned 90% of the biofilm cells non-viable at the concentration of 2 mg/ml. This extract was also more effective than the leaf extract (EEL2).

There was a difference between the effects of the extracts in different collections. Among the bark extracts, EEB2 was found to have greater antibiofilm capacity than EEB1, even at the lowest concentrations. Among the leaf extracts, the extracts from the first harvest (EEL1 and HEL1) had the greatest effect on the pre-formed biofilms.

Thus, as with biofilm formation, it is evident that one cannot consider phenological phase as the only determining factor influencing biological activity. The plant part and the solvent should also be taken into consideration to obtain a better action of the extracts.

Determination Of The Presence Of Gallic Acid By High Performance Liquid Chromatography (HPLC):

The HPLC analysis was performed in order to determine if there was a difference in the amount of gallic acid in the extracts obtained depending on the collection season. The presence of gallic acid was verified in the ethanolic extracts of leaves and bark, in both collections (Fig. 7). However, the leaf extract showed a lower concentration of gallic acid in the first harvest (0.0003 mg/ml) when compared to the second one (0.0023 mg/ml) (Fig. 7b). The concentration of gallic acid in the cuttings varied from 0.0160 mg/ml (harvest 1) and 0.0218 mg/ml (harvest 2), however this difference was not significant, indicating that this compound does not vary temporally in this part of the plant.

Discussion

One of the difficulties in fighting infections by *Candida* spp. is the ability of these microorganisms to form biofilms that hinders, and sometimes prevents, the action of antifungal drugs (CAVALHEIRO; TEIXEIRA, 2018). In addition, species of this genus are also able to change their yeast form (unicellular) to a pluricellular form, with formation of hyphae or pseudo hyphae (CIUREA et al., 2020) making it difficult for antifungals to enter the biofilm and making their action impossible.

The present study was conducted to understand if the effect of *C. leptophloeos* extracts on biofilms of *Candida* spp. varies depending on the time of collection of the plant, that is, its phenological phase. To this end, we evaluated how the extracts influenced the formation of biofilms and the development of pre-formed biofilms, using the colorimetric technique with crystal violet that allows the quantification of biofilm biomass.

Despite being a widely used technique within this field of research (COSTA et al., 2013), the analysis of the results obtained through the technique with crystal violet raised some questions, for example: (1) the OD values of the controls, for the most part, were below the OD values after action of the extracts, a result that was not expected, since in the controls there is no action of any inhibitory agent, leading us to suggest that the extracts stimulated the formation of biofilms in most treatments; (2) the expected relationship between extract concentration and the amount of fungal biomass was not observed, i.e., for most extracts, the higher the extract concentration, the higher was the fungal biomass, also leading us to suggest that the extracts stimulated biofilm formation; and finally, (3) the color of the extracts may have interfered in the reading of OD values in the spectrophotometer.

The fact that the strong color of the extracts, especially the ethanolic extracts of the leaves, hindered the reading of the results by spectrophotometer, despite the dilutions that were performed, was also observed in other studies dealing with the same plant species (DANTAS-MEDEIROS et al., 2021a).

Thus, this third question raised, made us perform the tests by means of the cell viability technique with methylene blue. This technique allows the quantification of non-viable yeast cells, once they do not present enzymatic activity, not being able to reduce the methylene blue dye. When analyzing the results obtained, it was noticed that all extracts presented inhibitory capacity, both in the formation of biofilms and in pre-formed biofilms.

There are several techniques that can be used to evaluate biofilms formed by *Candida* spp. Among them the viable cell count is one of the most used methods, including for analysis of the effectiveness of antifungal agents against biofilms of *Candida* spp. (COSTA et al., 2013).

After the analysis, it was noticed that the extracts of the second harvest of bark, in which the plant was with fruit formation, showed a more inhibitory effect on biofilms when compared to the extracts of the bark of the first harvest, when the plant was with leaves and flowers formation. As for the leaf extracts, the extracts from the first harvest showed better results.

Besides the ability to decrease the biofilm formation, the extracts were able to decrease the biomass of the biofilm already formed. This antimicrobial effect of *C. leptophloeos* barks and leaves was also observed by other authors (PEREIRA et al., 2017; PESSOA et al., 2021).

Commiphora leptophloeos extracts were also able to decrease the formation of pseudo hyphae of *C. albicans* URM 4387, *C. krusei* URM 6352 and *C. tropicalis* URM 4262, which is considered an important virulence factor of these yeasts (CAVALHEIRO; TEIXEIRA, 2018). Although clinical studies are needed, the fact that the extracts show this ability in the laboratory shows that the extracts have high antifungal capacity, being a promising source of compounds that can be used in the treatment of these infections.

Through the HPLC technique, the quantification of gallic acid was performed, which is a phenolic compound, known for its antioxidant and antibiofilm properties of bacteria and *C. albicans* (GIORDANI et al., 2020). It was observed that the presence of gallic acid in the ethanolic extracts of the barks was in greater amount than in the leaf extracts. The time of collection did not influence the amount of gallic acid in the bark extracts, however, the extract of the leaves from the second harvest showed a higher amount of the compound than the first harvest.

The presence of this compound is an indication of the potential of these extracts against biofilm, since this compound acts in suspending the adhesion of yeast cells to the surface (LAHIRI et al., 2019). Additionally, in a recent study, Liberato et al, (2022) studied the effects of gallic acid on fluconazole-resistant *Candida* spp. and

concluded that it acts in different ways on the cell of this yeast, e.g., interfering with membrane integrity as well as the transmembrane potential of mitochondria, causing cell apoptosis.

In the present study, gallic acid was observed in all ethanolic extracts of the leaves and bark, although in different amounts. This result corroborates with previous studies, which found gallic acid in the barks of *C. leptophloeos* (PEREIRA et al., 2017).

The barks of *C. leptophloeos* showed a higher amount of gallic acid than the leaves, in both collections. As for the leaves, it is observed that the amount of this compound was higher in the second collection. This indicates that the leaves tend to produce more gallic acid during the fruiting season.

Gallic acid has antifungal properties that may be important for the plant's defense against diseases caused by fungal pathogens. A recent study also reported variations in the amount of gallic acid in different months, which may be relating to the construction of the plant's chemical defense during its development (ZHOU et al., 2020).

It is evident that the biological activities, exerted by the secondary metabolites of plants, can be influenced by the phenological phase in which the plant is at the time of collection (ÇIRAK et al., 2007; GOBBO-NETO; LOPES, 2007).

When evaluating the effects of the extracts, from different phenological phases, on the pre-formed biofilms of *Candida* spp. the bark extracts prepared in the fruiting phase of the plant were slightly better. Despite being a small difference, these extracts showed a higher percentage of non-viable cells when compared to the first collection.

Differences in the number of metabolites depending on the phenological phase have been reported for other species (ÇIRAK et al., 2007; SOUZA DE OLIVEIRA et al., 2017), however, this is the first time that this study is conducted with *C. leptophleos*.

This species is characterized by starting the sprouting of leaves at the end of the dry season, so when the rainy season arrives the plant is already with fruits ready for dispersal (DE LIMA et al., 2012). This strategy may explain why the bark extracts of the second collection (rainy season) showed greater effect than those of the first, since, as the plant is already in its full development, it can then invest energy in the production of secondary metabolites that have biological activities.

However, for the leaves, the extracts from the first collection showed greater antibiofilm capacity. Thus, it is evident that the phenological phase is not the only factor to determine the biological activity of the plant. It is known that differences in the composition of secondary metabolites occur differently for each part of the plant (CHEN et al., 2012), since in the same season, parts of the plant tissues produce different metabolites in different amounts.

Thus, it is up to the researcher, to investigate which part of the plant offers in best result and in which phenological phase/season of collection this result is optimized.

Conclusion

The biological activities of plants are the result of the action of secondary metabolites produced, which are influenced by the physiology of the plant. Thus, determining the time of collection of a botanical material is crucial for the development of extracts with their maximum capacity. Thus, we tested whether there was an

influence of the phenological phases (vegetative and reproductive) of *Commiphora leptophloeos* on biofilms of *Candida* spp. We noticed that there was a slight difference between the effect of *C. leptophloeos* leaf and bark extracts on biofilms of *Candida* spp. When analyzing the presence of gallic acid in the extracts, we noticed that, although the bark presented a higher amount of gallic acid, there was no significant difference between the harvests. However, this difference is noticeable in the leaf extracts. These results indicate that, depending on the researcher's objective, the time of collection and the part of the plant to be used are important factors to be considered to obtain the best results for a certain biological activity, as well as higher concentrations of the desired compound.

Declarations

Competing interests: The authors declare no competing interests.

Author's contribution:

SILVA, AGR was responsible for conceptualization, methodology, investigation, design of the experiments, and the writing of the original draft. SIQUEIRA, VM was responsible for conceptualization, methodology, investigation and writing review of the manuscript. LIMA, ALA was responsible for conceptualization, supervision, project administration and writing review.

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Tables

Table 1. Differences in climatic conditions between the haversts of *Commiphora leptophloeos* in Serra Talhada, PE, Brazil. The first harvest was realized at the end of the dry season (Dec/2020), and the second one was realized at the rain season (Mar/2021).

Harvest	First (1)	Second (2)
Date	12/10/2020	03/04/2021
Time	08:00-11:00 am	08:00-11:00 am
Average temperature	28°C	27°C
Average humidity	50%	60%
Rain index (for the day)	0.0 mm	0.0 mm
Total rainfall (of the month)	1.7 mm	186.8 mm

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) in mg/ml, of ethanolic and hexanolic extracts of the leaves (EEL and HEL) and ethanolic extracts of the bark (EEB) of *Commiphora leptophloeos* collected in Dec/2020 (Harvest 1) and Mar/2021 (Harvest 2) against *Candida* spp. strains. All tests were performed in triplicates.

Extract	Yeast					
	C. albicans URM 4387		*C. krusei* URM 6352		*C. tropicalis* URM 4262	
	MIC	MFC	MIC	MFC	MIC	MFC
	(mg/ml)		(mg/ml)		(mg/ml)	
EEL1	2	>2	1.5	2	1.5	2
HEL1	1.75	2	1.5	2	1.5	2
EEB1	1.75	2	1.5	2	1.5	2
EEL2	2	>2	1.5	2	1	2
HEL2	2	>2	1.5	2	1.5	2
EEB2	>2	>2	1.5	2	1.5	2

Table 3. Influence of leaf extracts and barks of *Commiphora leptophloeos* on the formation of biofilms of *C. albicans* URM 4387 by methylene blue staining technique. The values indicate the percentage of non-viable planktonic cells of *C. albicans* URM 4387 after action of different extracts of *C. leptophloeos*. The values are expressed by the arithmetic mean of the triplicates. The symbols indicate statistical differences in the percentage of viable cells compared to the control for each extract. * = $p < 0.05$; **= $p < 0.01$; *** = $p < 0.001$.

	Extract concentration (mg/ml)						Extract concentration (mg/ml)				
EEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEK2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	NV	55,3***	44,3***	22,3**	10,6	Mean	NV	79,6***	65,0	54,0**	36,0
SD		±3,6	±1,2	±2,4	±3,0	SD		±2,0	±5,3	±0,8	±4,5
EEB1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEB2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	91,3	84,6**	72,0	67,6*	54,3	Mean	81,0	73,3	65,3	50,3*	45,3
SD	±6,0	±6,1	±6,5	±8,5	±6,6	SD	±1,7	±4,7	±1,1	±4,1	±3,5
HEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**HEL2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	84,6*	77,6***	72,6***	66,0***	54,6	Mean	80,0	64,6*	58,6***	57,6**	44,6
SD	±3,5	±3,7	±3,2	±1,7	±1,5	SD	±2	±3,2	±1,1	±1,5	±0,5

SD = standard deviation; NV = not visible; EEL1- Ethanolic extract of leaves – harvest 1; EEL2 - Ethanolic extract of leaves – Harvest 2; EEB1 - Ethanolic extract of bark – harvest 1; EEB2 - Ethanolic extract of bark – harvest 2; HEL1 – Hexanic extract of leaves – harvest 1; HEL2 – Hexanic extract of leaves – harvest 2.

Table 4. Influence of leaf extracts and barks of *Commiphora leptophloeos* on the formation of biofilms of *C. krusei* URM 6352 by methylene blue staining technique. The values indicate the percentage of non-viable planktonic cells of *C. krusei* URM 6352 after action of different extracts of *C. leptophloeos*. The values are expressed by the arithmetic mean of the triplicates. The symbols indicate statistical differences in the percentage of viable cells compared to the control for each extract. * = p <0.05; **= p <0.01; *** = p<0.001.

	Extract concentration (mg/ml)						Extract concentraton (mg/ml)				
EEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEL2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	NV	75,0*	68,6***	56,3**	42,3	Mean	NV	86,6***	61,3***	50,6***	36,6
SD		±5,2	±2,3	±1,1	±1,5	SD		±1,5	±3,2	±2,5	±2,8
EEB1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEB2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	96,3***	87,3	79,6	45,0**	32,3	Mean	93,6***	70,0***	60,0+	50,6*	40,3
SD	±1,5	±3,0	±4,1	±6,0	±3,0	SD	±3,5	±2,0	±2,6	±3,7	±3,5
HEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**HEL2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	94,6**	88,3***	64,6	53,3**	42,3	Mean	81,3	67,3	55,6***	44,3***	32,3
SD	±2,0	±3,5	±0,5	±1,5	±2,0	SD	±2,3	±3,0	±2,0	±3,5	±1,1

SD = standard deviation; NV = not visible; EEL1- Ethanolic extract of leaves – harvest 1; EEL2 - Ethanolic extract of leaves – Harvest 2; EEB1 - Ethanolic extract of bark – harvest 1; EEB2 - Ethanolic extract of bark – harvest 2; HEL1 – Hexanic extract of leaves – harvest 1; HEL2 – Hexanic extract of leaves – harvest 2.

Table 5. Influence of leaf extracts and barks of *Commiphora leptophloeos* on the formation of biofilms of *C. tropicalis* URM 4262 by methylene blue staining technique. The values indicate the percentage of non-viable planktonic cells of *C. tropicalis* URM 4262 after action of different extracts of *C. leptophloeos*. The values are expressed by the arithmetic mean of the triplicates. The symbols indicate statistical differences in the percentage of viable cells compared to the control for each extract. * = p <0.05; **= p <0.01; *** = p<0.001.

	Extract concentration (mg/ml)					Extract concentation (mg/ml)					
EEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEL2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	NV	86,3***	79,0	63,0**	51,6	Mean	NV	89,3	74,0***	61,0***	57,0
SD		±2,0	±1,0	±3,6	±3,0	SD		±2,5	±3,6	±2,6	±2,0
EEB1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEB2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	91,3***	80***	76,6***	41,6**	19,6	Mean	91,6***	69,3	50,3	47,0***	34,6
SD	±3,2	±2	±1,5	±2,5	±2,0	SD	±5,6	±2,0	±4,0	±1,7	±3,5
HEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**HEL2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	87,6	83,3	66,6	58,6	49,0	Mean	82,0	70,6***	53,6*	46,6***	39,3
SD	±0,5	±3,7	±0,5	±3,0	±2	SD	±2,6	±2,0	±1,1	±2,5	±2,5
SD = standard deviation; NV = not visible; EEL1- Ethanolic extract of leaves – harvest 1; EEL2 - Ethanolic extract of leaves – Harvest 2; EEB1 - Ethanolic extract of bark – harvest 1; EEB2 - Ethanolic extract of bark – harvest 2; HEL1 – Hexanic extract of leaves – harvest 1; HEL2 – Hexanic extract of leaves – harvest 2.											

Table 6. Influence of leaf extracts and barks of *Commiphora leptophloeos* on pre-formed biofilms of *C. albicans* URM 4387 by methylene blue staining technique. The values indicate the percentage of non-viable planktonic cells of *C. albicans* URM 4387 after action of different extracts of *C. leptophloeos*. The values are expressed by the arithmetic mean of the triplicates. The symbols indicate statistical differences in the percentage of viable cells compared to the control for each extract. * = p <0.05; **= p <0.01; *** = p<0.001.

	Extract concentration (mg/ml)						Extract concentration (mg/ml)				
EEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEL2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	NV	52,0**	34,6***	19,6**	10,6	Mean	NV	71,0**	50,0*	42,6**	28,6
SD		±2,6	±2,8	±2,08	±1,5	SD		±3,6	±2,6	±4,1	±2,5
EEB1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEB2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	87,3	80	62,3	58,0	47,3	Mean	75,0	69,0	59,6**	51,0	39,3
SD	±4,7	±2,6	±4,7	±4,3	±2,0	SD	±5,0	±2,6	±4,7	±4,0	±3,2
HEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**HEL2**	2	1	0,5	0,250	0,125
Mean	77,6	71,0	58,0	39,0**	20,6	Mean	67,6***	64,6*	55,6	29,3***	18,0
SD	±1,1	±1,7	±3,0	±1,7	±3,2	SD	±0,5	±3,2	±3,0	±2,5	±3,0

SD = standard deviation; NV = not visible; EEL1- Ethanolic extract of leaves – harvest 1; EEL2 - Ethanolic extract of leaves – Harvest 2; EEB1 - Ethanolic extract of bark – harvest 1; EEB2 - Ethanolic extract of bark – harvest 2; HEL1 – Hexanic extract of leaves – harvest 1; HEL2 – Hexanic extract of leaves – harvest 2.

Table 7. Influence of leaf extracts and barks of *Commiphora leptophloeos* on pre-formed biofilms of *C. krusei* URM 6352 by methylene blue staining technique. The values indicate the percentage of non-viable planktonic cells of *C. krusei* URM 6352 after action of different extracts of *C. leptophloeos*. The values are expressed by the arithmetic mean of the triplicates. The symbols indicate statistical differences in the percentage of viable cells compared to the control for each extract. * = p <0.05; **= p <0.01; *** = p<0.001.

	Extract concentration (mg/ml)						Extract concentration (mg/ml)				
EEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEL2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	NV	70,3	63,0**	44,3***	33,6	Mean	NV	73,0***	51,3**	32,3+	17,3
SD		±3,2	±2,0	±3,5	±1,1	SD		± 3,5	±1,8	±2,4	± 1,6
EEB1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEB2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	80	63,3	59,6***	35,3*	22,3	Mean	90,0***	76,3*	61,0*	35,3*	29,3
SD	±3	±5,5	±2,5	±2,5	±2,3	SD	±1,0	±1,1	±1,7	±2,5	±2,0
HEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**HEL2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	65,6*	52,6***	35,0***	32,6***	21,3	Mean	59,0***	54,0**	47,3**	34,3**	14,3
SD	±2,5	±3,7	±2,0	±3,5	±2,0	SD	±4,0	±3,0	±2,0	±0,5	±2,0

SD = standard deviation; NV = not visible; EEL1- Ethanolic extract of leaves – harvest 1; EEL2 - Ethanolic extract of leaves – Harvest 2; EEB1 - Ethanolic extract of bark – harvest 1; EEB2 - Ethanolic extract of bark – harvest 2; HEL1 – Hexanic extract of leaves – harvest 1; HEL2 – Hexanic extract of leaves – harvest 2.

Table 8. Influence of leaf extracts and barks of *Commiphora leptophloeos* on pre-formed biofilms of *C. tropicalis* URM 4262 by methylene blue staining technique. The values indicate the percentage of non-viable planktonic cells of *C. tropicalis* URM 4262 after action of different extracts of *C. leptophloeos*. The values are expressed by the arithmetic mean of the triplicates. The symbols indicate statistical differences in the percentage of viable cells compared to the control for each extract. * = p <0.05; **= p <0.01; *** = p<0.001.

	Extract concentration (mg/ml)						Extract concentration (mg/ml)				
EEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEL2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	NV	74,6*	56,6***	53,6**	40	Mean	NV	67,3***	62,0***	38,6	16,3
SD		±3,2	±4,7	±4,7	±2,6	SD		±4,5	±3,4	±6,0	±3,0
EEB1	**2**	**1**	**0,5**	**0,250**	**0,125**	**EEB2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	87,3**	76,6	58,0***	30,3***	17,3	Mean	87,3***	58,6***	51,6	42,6*	19,3
SD	±2,0	±0,5	±4,0	±2,5	±3,2	SD	±2,5	±3,2	±5,7	±0,5	±4,5
HEL1	**2**	**1**	**0,5**	**0,250**	**0,125**	**HEL2**	**2**	**1**	**0,5**	**0,250**	**0,125**
Mean	64,3	58,3***	50,3*	43,6**	19,6	Mean	61,0***	58,6***	41*	32,3*	15,3
SD	±4,0	±7,0	±4,0	±3,0	±2,0	SD	±3	±0,5	±2,0	±3,0	±2,8

SD = standard deviation; NV = not visible; EEL1- Ethanolic extract of leaves – harvest 1; EEL2 - Ethanolic extract of leaves – Harvest 2; EEB1 - Ethanolic extract of bark – harvest 1; EEB2 - Ethanolic extract of bark – harvest 2; HEL1 – Hexanic extract of leaves – harvest 1; HEL2 – Hexanic extract of leaves – harvest 2.

Figures



Figure 1

Commiphora leptophloeos collected in Serra Talhada/PE. (A) Harvest 1: Dec/2020 - plant in vegetative phenophase; (B) Harvest 2: Mar/2021 - plant with fruits.

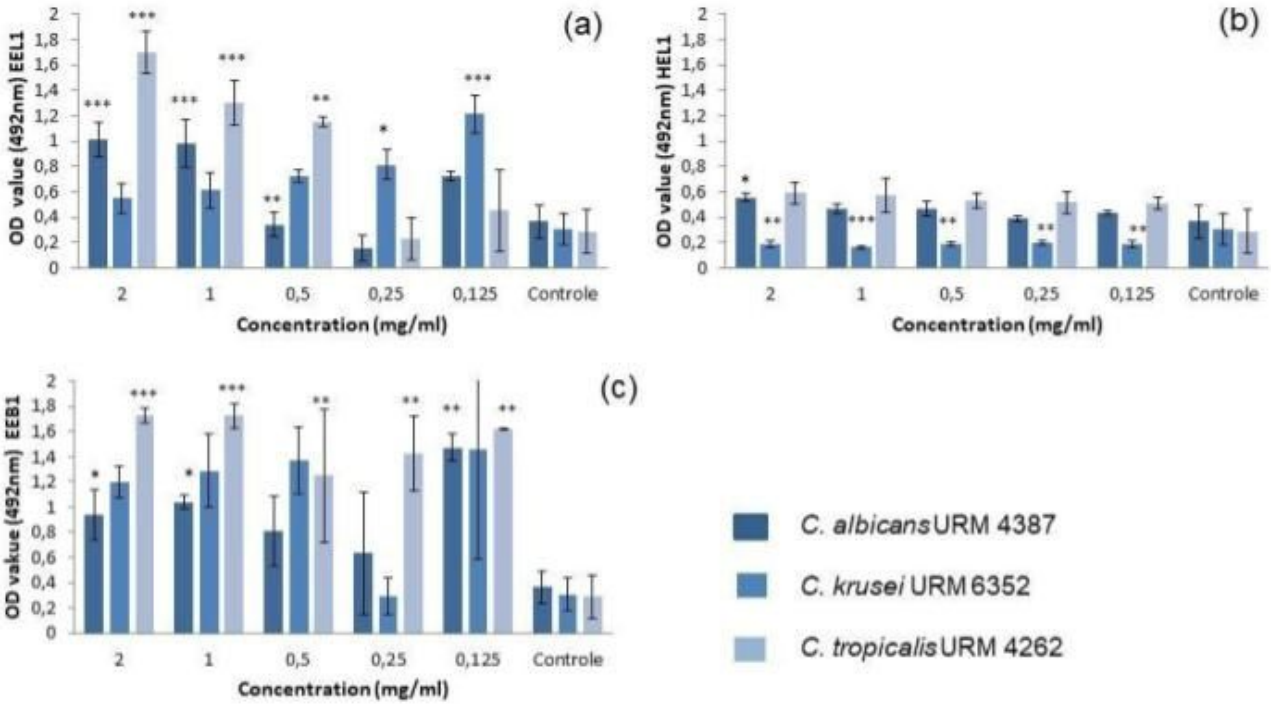


Figure 2

Optical Density (OD) values, at 492 nm of ethanolic (a) and hexanolic (b) extracts of the leaves (EEL and HEL) and ethanolic extracts of the barks (c) (EEB) of *C. leptophloeos*, collected in Dec/2020 (harvest 1), on biofilm formation of *Candida* spp. The symbols on the bars indicate statistical differences between the mean OD values compared to the control for each *Candida* spp. strain. * = p <0.05; ** = p <0.01; *** = p<0.001.

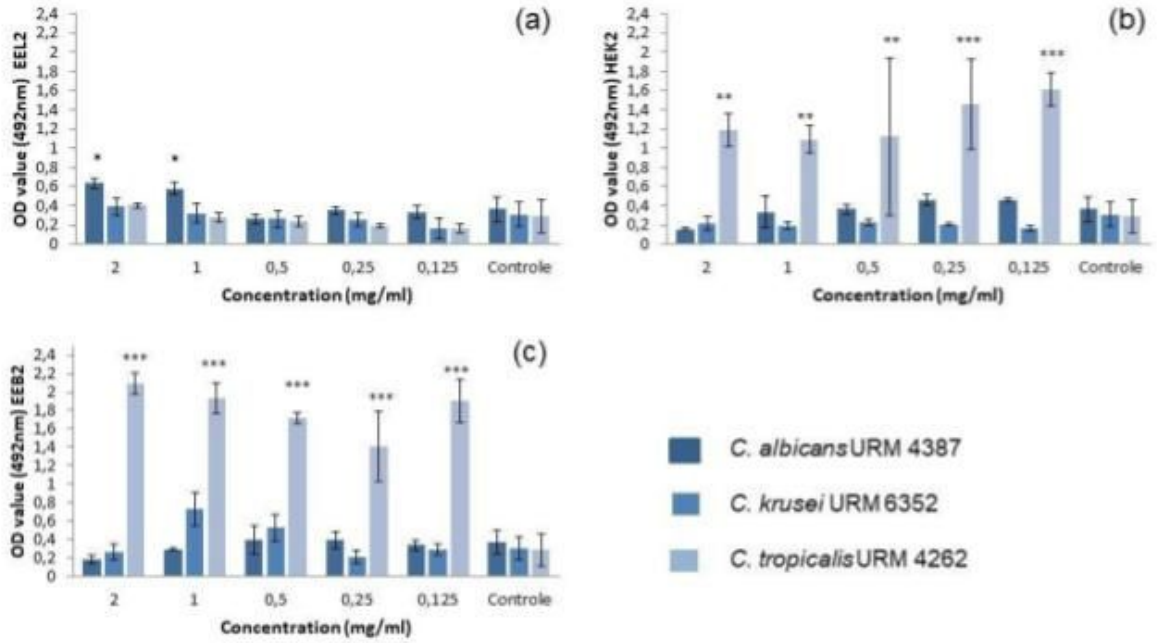


Figure 3

Optical densities (OD) values, at 492 nm of ethanolic (a) and hexanic (b) extracts of the leaves (EEL and HEL) and ethanolic extracts of the barks (c) (EEB) of *Commiphora leptophloeos*, collected in Mar/2021 (harvest 2), on biofilm formation of *Candida* spp. The symbols on the bars indicate statistical differences of the mean OD values compared to the control for each *Candida* spp. strain. * = p <0.05; **= p <0.01; *** = p<0.001.

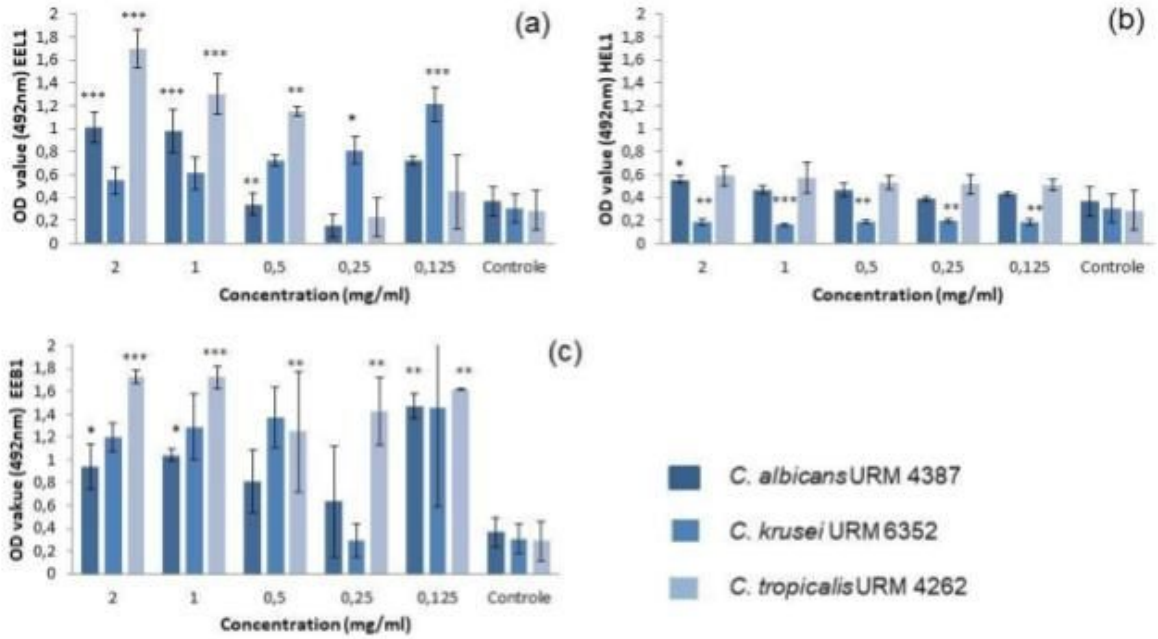


Figure 4

Optical Density (OD - 492 nm) values of ethanolic (a) and hexanic (b) extracts of the leaves (EEL and HEL) and ethanolic extracts of the barks (c) (EEB) *Commiphora leptophloeos*, collected in Dec/2020 (harvest 1), on pre-formed biofilms of *Candida* spp. The symbols on the bars express statistical differences of the mean OD values compared to the control for each *Candida* spp. strain. * = p <0.05; ** = p <0.01; *** = p<0.001.

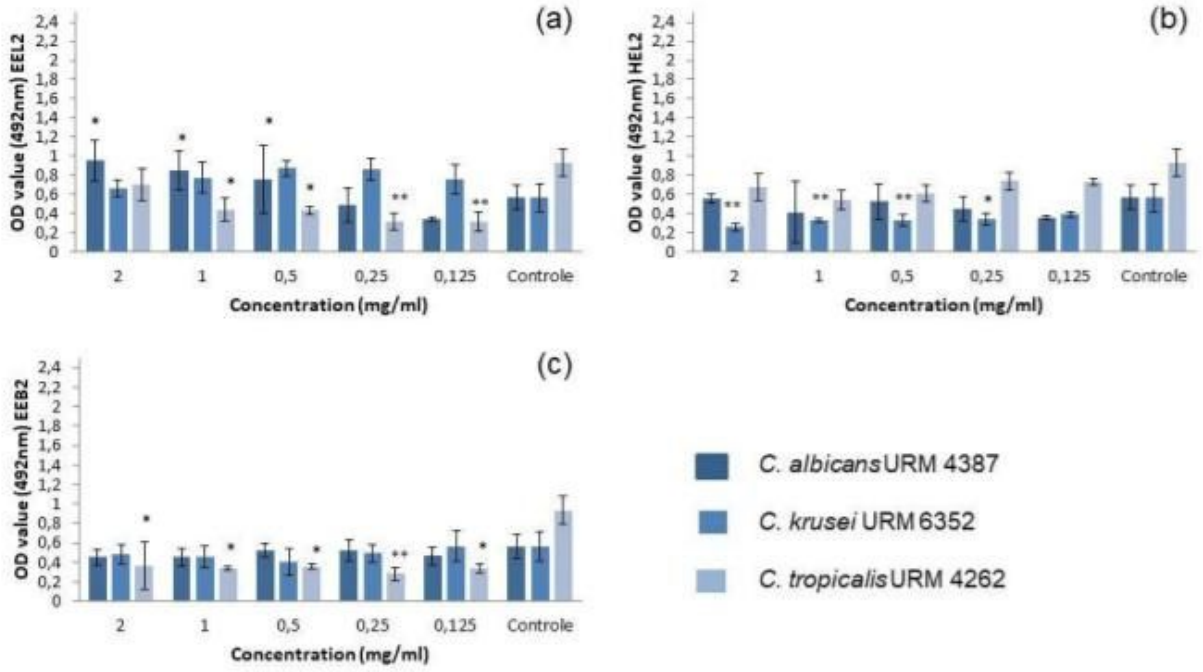


Figure 5

Optical Density (OD) values of ethanolic (a) and hexanic (b) extracts of the leaves (EEL and HEL) and ethanolic extracts of the barks (c) (EEB) of *Commiphora leptophloeos*, collected in Mar/2021 (harvest 2), on pre-formed biofilms of *Candida* spp. The symbols on the bars indicate statistical differences of the mean OD values compared to the control for each *Candida* spp. strain. * = p <0.05; ** = p <0.01; *** = p<0.001.

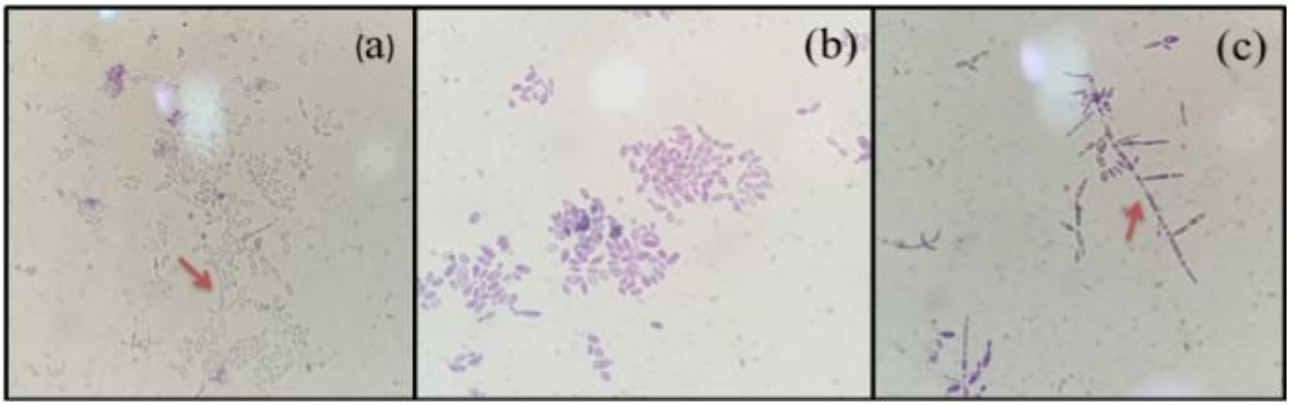


Figure 6

Effects of the extracts on the morphology of *Candida* spp. yeasts. The arrows indicate the pseudo hyphae. It is observed that in (a) the pseudo hyphae are less evident than in (c). (a) Ethanolic Extract of the Barks of harvest 2 - EEB2 (0.125mg/ml) against *C. krusei* URM 6352. (b) Hexanic Extract of the Leaves of harvest 2 - HEL2 (0.125mg/ml) against *C. krusei* URM 6352. (c) Control of *C. krusei* URM 6352.

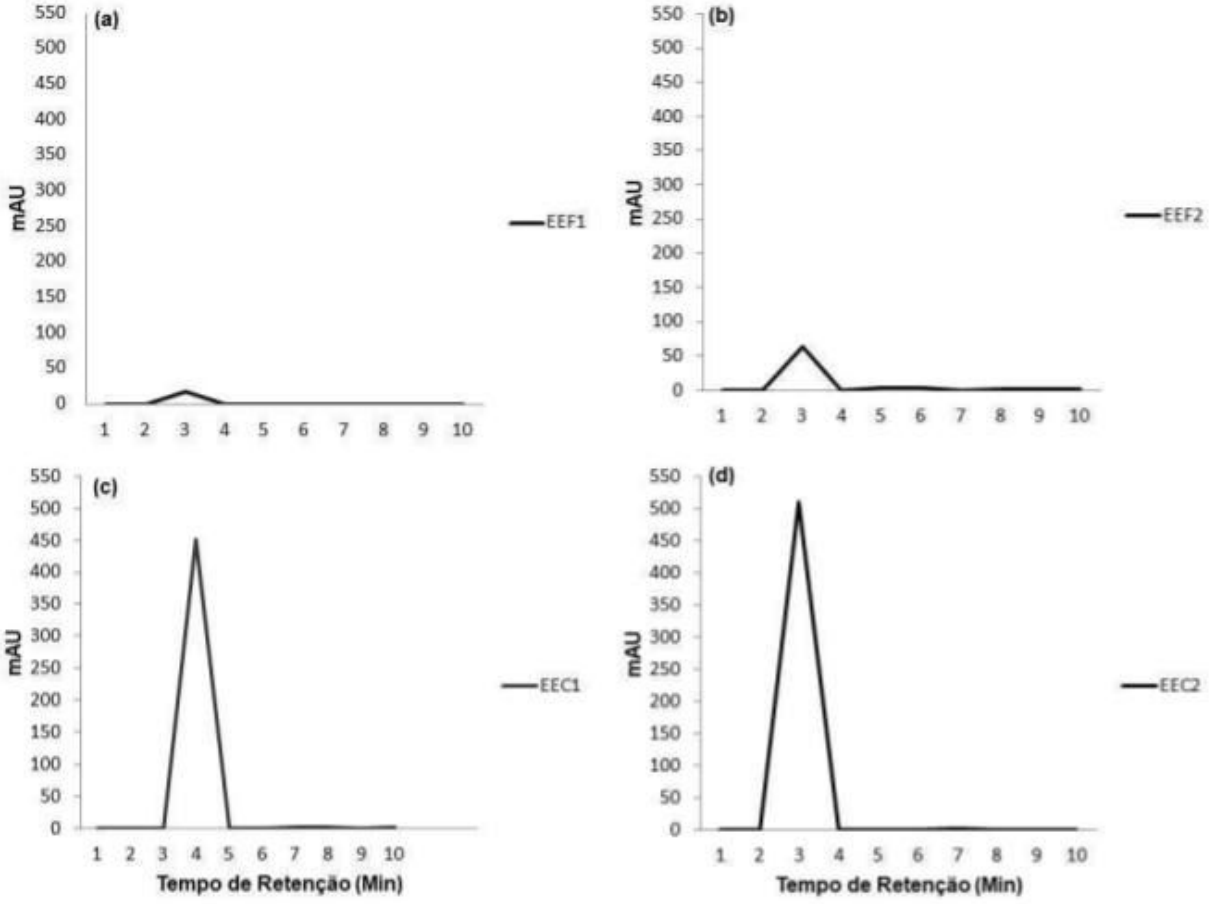


Figure 7

Presence and quantification of gallic acid in ethanolic extracts of the leaves (EEL) and bark (EEB) of *Commiphora leptophloeos* collected in Dec/2020 (harvest 1) and Mar/2021 (harvest 2). a) EEL1; b) EEL2; c) EEB1; d) EEB2.