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BACTERIAL AND FUNGAL PATHOGENESIS - RESEARCH PAPER

Effects of *Euterpe oleracea* Mart. extract on *Candida* spp. biofilms

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Abstract

Problem of research *Candida* spp. biofilms are complex microbial communities that have been associated with increasing resistance to clinically available antifungal drugs. Hence, novel pharmacological approaches with ability to inhibit biofilm formation have been investigated.

Aim of study The aim was to analyze in vitro antifungal activity of *Euterpe oleracea* Mart. (açaí berry) extract on biofilm strains of *Candida albicans*, *C. parapsilosis*, and *C. tropicalis* that were formed on abiotic surfaces.

Remarkable methodology Biofilms of *C. albicans*, *C. parapsilosis*, and *C. tropicalis* were grown in vitro. They were then treated with *E. oleracea* Mart. extract at different concentrations (7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/mL) for evaluation of both biofilm removal and anti-biofilm activity.

Remarkable results All *Candida* species analyzed formed biofilms on abiotic surfaces. Yet, increased biofilm formation was displayed for *C. tropicalis* in comparison with the other two species. *E. oleracea* Mart. extract was shown to inhibit biofilm formation at all concentrations used when compared to no treatment ($p < 0.05$).

Significance of the study In the current study, the extract of *E. oleracea* Mart. demonstrated antifungal activity against *Candida albicans*, *C. parapsilosis*, and *C. tropicalis* biofilms, regardless of the dose utilized. These results are important to evaluate a natural product as antifungal for *Candida* species.

Keywords *Euterpe oleracea* · Candida · Biofilms · Antifungal activity

Abbreviations

ATCC	Anatomical Therapeutic Chemical Code
BHI	Brains heart infusion
ESI	Electrospray ionization
ESI-FT-ICR MS	Cyclotron analyzer coupled to a Fourier transform
ICMBio	Instituto Chico Mendes de Conservação da Biodiversidade
Sisgen	Sistema Nacional de Gestão do Patrimônio Genético

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Introduction

Infections by *Candida* species, such as *Candida albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*, are increasingly frequent [1–3]. They often occur due to host immunosuppression and/or virulence factors expressed by these yeasts, which contribute to their ability to colonize, penetrate, and invade tissues [4]. Such strains have also the ability to form biofilm.

According to Costerton (1999), biofilm consists of cells that attach to surfaces aggregate in a hydrated polymeric matrix of their own synthesis to form biofilms [5].

Importantly, biofilms may hinder the penetration of most antifungal agents, thereby leading to a poor response to treatment and drug resistance [6–9]. The formation and development of distinct phases of biofilm (adhesion/colonization, maturation, and dispersion) are mediated by regulatory genetic alterations and complex molecular events [10].

Because they consist in a matrix of microorganisms, biofilms can be considered a defense strategy of pathogens, affecting either biotic or abiotic surfaces such as medical devices (e.g., catheters, bladder probes) [11–13].

Moreover, biofilms have been increasingly linked to both mucosal infections such as candidiasis, which is facilitated by virulent factors from *Candida* species, including their capacity to form biofilms and the transition to filamentous or hyphal form [14].

In this context, natural products with ability to inhibit or disrupt biofilms have been investigated as a potential source of novel antifungals [15, 16]. Al-Sokari and Sheikha (2015) evidenced that crude extracts of *Ruta graveolens* L have good inhibition zone against *Escherichia coli* and *Pseudomonas aeruginosa*. Latex of *Ficus carica* Linn plants showed that it is a good inhibitor for *Candida albicans* [17].

Khan and Ahmad had studied the inhibitory effect of essential oil of *Cymbopogan citratus* and *Syzygium aromaticum* on the biofilm of drug-resistant *Candida* from clinical origin. *C. citratus* was capable to inhibit the biofilm formation of approximately 88% in *C. albicans* 04 and 82% in *C. albicans* SC5314 and *S. aromaticum* inhibited 52% and 57% biofilm in above-mentioned test strains at the same concentration [18].

Açaí (*Euterpe oleracea* Mart.) is a native plant from Amazonian region, and it has been used despite of its high antioxidant activity as antimicrobial. *Euterpe* genus includes over 28 species distributed throughout the Amazon region in Latin America, where *E. oleracea*, *E. precatoria*, and *E. edulis* are the most frequent species.

The phytochemical composition of the fruit known as "açaí berry" has been well characterized. It includes phenolic acids, anthocyanins (e.g., cyanidin-3-rutinoside and cyanidin-*O*-glucoside), proanthocyanidins, lignans (e.g., aryltetrahydronaphthalene, dihydrobenzofuran, furofuran, 8-*O*-4′-neolignan, and tetrahydrofuran), and polyphenolic constituents (e.g., epicatechin, the catechin homoorientin, orientin, isovitexin, and taxifolin deoxyhexose) [19].

Hence, the present study was aimed at investigating in vitro whether the treatment with *E. oleracea* Mart. extract would have the ability to inhibit or disrupt biofilms from *C. albicans*, *C. parapsilosis*, and *C. tropicalis* formed on abiotic surfaces.

It is important to study natural products as antifungal agents, considering the remarkable resistance of *Candida* species to imidazoles.

This is relevant to have alternative treatments because the increasing incidence of drug-resistant pathogens and the toxicity of existing antifungal compounds have increased interest in the antifungal properties of natural products. Furthermore, most of the available antifungals are either ineffective against Candida biofilms or exhibit their inhibitory activity at high concentrations.

Methods

Microbial strain identification

Commercially available strains of *C. albicans* (ATCC 10,231), *C. tropicalis* (ATCC 1369), and *C. parapsilosis* (ATCC 22,019) were obtained from Plast Labor (Rio de Janeiro, RJ, Brazil) and kept under refrigeration until use. Field experiments have been approved by the Brazilian Ministry of the Environment, Instituto Chico Mendes de Conservação da Biodiversidade—ICMBio (approval number: 57805–1).

The strains were kept on Sabouraud Dextrose Agar with 4% chloramphenicol. The fungal suspension for the experiments was prepared from 24 h colonies diluted in 0.85% saline according to the 0.5 MacFarland scale ($1–5 \times 10^6$ cells/mL).

Preparation of E. oleracea Mart. extract

The fruits of *E. oleracea* Mart. used in this study were collected from Juçara Park, located in São Luís, MA, Brazil (latitude: 02° 31′ 47″ S, longitude: 44° 18′ 10″ W, altitude: 24 m). Approval was obtained through the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen, protocol A91B0BA). The extract was prepared using a protocol previously utilized with some adaptations [20]. Briefly, fruits were thawed and washed three times with distilled water, then soaked in warm water for 1 h. Subsequently, 365 g of whole fruit extract was grinded and mixed with 400 mL of ethyl alcohol p.a. For 10 days, the mix was shaken for 2 h/day, followed by vacuum filtration. The solvent was removed by rotary evaporation, lyophilized, aliquoted, and then refrigerated until use. After the maceration period, the extract was concentrated in rotoevaporator, lyophilized, and sent for identification of chemical compounds through mass spectrometry analysis.

Mass spectrometry analysis

Mass spectrometry analysis was performed using an electrospray ionization (ESI) source and a cyclotron analyzer coupled to a Fourier transform (ESI-FT-ICR MS). Samples were diluted with 0.1% acetic acid for positive analysis,

and the resulting solution was then infused directly into the SOLARIX 9.4 T mass spectrometer (Bruker Daltonics, Bremen, Germany), operating in a range of 100–1000 m/z. The general conditions for EIS analysis were gas pressure of 0.3 psi, capillary voltage of 4.5 kV, and 220 °C for the ion transfer capillary temperature. The ESI (+)—FT—ICR MS spectra were acquired and processed using the Compass Data Analysis software (Bruker Daltonics).

Evaluation of biofilm formation

Biofilm formation was evaluated in 96-well polystyrene microplates as previously described [21]. First, *Candida* spp. strains were cultivated in Sabouraud Dextrose Agar and incubated at 37 °C for 24 h. Next, isolates were diluted in saline solution to match the 0.5 McFarland turbidity standard, corresponding to 1×10^6 to 5×10^6 cells per mL [22]. The wells of microplates were filled sequentially in triplicates. In the negative control group, only 200 µL of BHI with 6% glucose were added. In the remaining wells, 180 µL of BHI with 6% glucose plus 20 µL of the suspension of each *Candida* species in saline solution was added. The microplates were incubated (37 °C, 24 h) and, subsequently, washed three times with sterile distilled water, and received 200 µL of crystal violet dye each well for 5 min. They were then washed three times with sterile distilled water, and lastly, 200 µL of sterile distilled water was added to each well for spectrophotometric analysis at 570 nm wavelength absorbance.

Adhesion and antibiofilm activity of E. oleracea Mart. extract on Candida spp.

Biofilms in 96-well polystyrene microplates were formed by inoculum of *C. albicans*, *C. parapsilosis* and *C. tropicalis* (100 µL), incubated for 1 h and 30 min at 37 °C, which corresponds to adhesion phase of yeasts to the abiotic surface [21].

Subsequently, the medium was aspirated, the wells were washed three times with PBS 1X and 200 µL of different concentrations (7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/mL) diluted in BHI with 6% glucose were added. The wells were washed with sterile PBS 1X 3 times, fixed with PA ethanol for 2 min and stained with 1% violet crystal solution for 5 min and again washed with sterile 1X PBS 5 times to remove excess dye.

After this step, 200 µL of sterile 1X PBS was added to each well and the reading was performed on the Epoch microplate reader with a wavelength of 570 nm. In columns A1, A2, and A3 the positive control was inoculated adding only the culture medium.

Biofilm removal activity of E. oleracea Mart. extract on Candida spp. biofilms

To analyze in vitro biofilm removal activity of *E. oleracea* Mart. extract on biofilms formed by *C. albicans*, *C. parapsilosis*, and *C. tropicalis* on abiotic surfaces of microplates (TPP) after 72 h, different concentrations (7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/mL) of *E. oleracea* Mart. extract (200 µL) were added in each well over the mature biofilm [21].

Subsequently, the wells were washed with sterile 1X PBS 3 times, fixed with PA ethanol for 2 min and stained with 1% violet crystal solution for 5 min and again washed 5 times with 1X PBS to remove excess dye. After this step, 200 µL of sterile 1X PBS was added to each well and the reading was performed on the Epoch microplate reader with a wavelength of 570 nm.

Antibiofilm activity of E. oleracea Mart. extract in coverslips

The biofilm evaluation in coverslips was performed using protocols previously reported with some adaptations [23–25]. *C. albicans*, *C. tropicalis*, and *C. parapsilopsis* yeasts were grown in a 24-well plate containing 13 cm round glass coverslip in 1-mL 0.85% saline.

The fungal samples were seeded in Sabouraud Dextrose Agar and incubated at 37 °C for 24 h. Next, isolates were diluted in saline solution to match the 0.5 McFarland turbidity standard, corresponding to 1×10^6 to 5×10^6 cells per mL [22]. In each well, 100 µL of the fungal suspension was added and placed to adhere for 6 h. The wells were subsequently washed three times with 0.85% saline solution and then 1 mL of each concentration of the extract (7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/mL) was added, and plates were incubated for 48 h.

Next, the plates were incubated at 37 °C for 48 h in a BOD oven. After that, the coverslips were gently removed, washed with PBS 1X 3 times, dried at room temperature, fixed with PA ethanol, stained with 1% violet crystal solution. The coverslips were glued with mesh onto the surface of a glass slide and analyzed under the Ninkon optical microscope.

Cells adhered to the coverslip were counted and classified according to cell arrangement patterns adhered to glass coverslips as: diffuse pattern, when yeast cells adhered to entire surface of the glass coverslip without forming cell groups; localized adhesion, when involving groups of yeast that adhered to localized regions of the coverslip; aggregative, which is characterized by yeast clumps arranged as "stacked bricks" or "grape clusters" that attached to the glass slide surface. The formation of filaments or pseudohyphae along

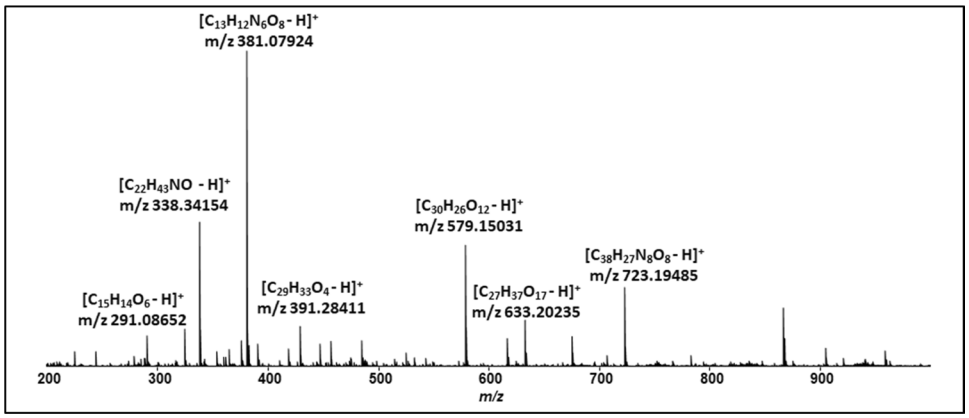


Fig. 1 Mass spectrometry from *Euterpe oleracea* Mart extract

Table 1 Compounds isolated from *Euterpe oleracea* Mart extract

m/z	Compounds
291,08,652	Epicatechin
338,34,154	Erucamide
381,07,924	N-(3-methoxy-5-nitrophenyl)-2-(5-methyl-3,4-dinitro-1H-pyrazol-1-yl) acetamide
391,28,411	Not identidied
579,15,031	Procyanidin B3
633,20,235	(4-Acetoxy-5-((2-((4.5-dihyroxytetrahydro-2H-pyran-2-yl) oxy)-4.5-dilydroxytetrahydro-2H-pyran-3-yl) oxy)-3-hydroxytetrahydrofuran-2-yl) methyl (E)-3-(4-hydroxy-3-methoxyphenyl) acrylate
723,19,485	Not identified

the surface of the coverslip characterized the filamentous or pseudohyphal pattern.

Statistical analysis

Data were presented as means ± standard deviations or as medians and interquatile ranges. Normality of variables was analyzed using the Shapiro–Wilk test. Comparisons were performed using the Kruskal–Wallis or Wilcoxon tests. A p value ≤ 0.05 was considered to be statistically significant. Statistical analyzes were performed using STATA (StataCorp, Release 14; College Station, TX, USA).

Results

Figure 1 displays the chemical characterization of crude ethanolic extract of açaí berry fruit by mass spectrometry analysis using the positive method. The extract was shown to be rich in polyphenols, and the compounds identified are exhibited in Table 1.

Further, *Candida* spp. used in this study had the ability to form biofilms on abiotic surfaces. Higher biomass formation on abiotic surfaces was observed in *C. tropicalis* (2.397 ± 0.23) and *C. parapsilosis* (1.176 ± 0.37) biofilms, whereas lower biomass was shown for *C. albicans* biofilm (0.53 ± 0.07).

Table 2 Median absorbance (570 nm) of *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis* species during biofilm formation, pre- and postuse of *E. oleracea* Mart extract

Strains	Absorbance		p value**
	Pre (n = 72)	Post (n = 72)	
	Median [IQR*]	Median [IQR*]	
C. albicans	0.773 [0.625–1.072]	0.058[0.049–0.081]	< 0.001
C. parapsilosis	1.504 [1.113–1.680]	0.049[0.047–0.053]	< 0.001
C. tropicalis	2.500 [2.332–2.587]	0.125 [0.056–0.333]	< 0.001

*Interquartile range (p25– p75)

**Wilcoxon test for paired samples

A variation in biomass formed between *Candida* species was observed, where *C. tropicalis* was the most adherent, thereby producing more biofilm. Table 2 shows the absorbance before and after the addition of *E. oleracea* Mart. extract to surfaces containing biofilms formed by each *Candida* species analyzed. It was found a statistically significant difference between the median absorbance measured before and after treatment with *E. oleracea* Mart. extract for all *Candida* species evaluated (p < 0.001).

Table 3 Absorbance for different concentrations of *E. oleracea* Mart extract, pre- and postuse, on biofilms of *C. albicans*, *C. parapsilosis*, and *C. tropicalis* on abiotic surfaces

Concentration (µg/mL)	Absorbance		*p* value**
	Pre ($n=72$)	Post ($n=72$)	
	Median [IIQ*]	Median [IIQ]	
7.8	1.341[0.765–2.538]	0.047 [0.046–0.055]	0.019
15.6	1.112 [0.812–2.489]	0.051 [0.049–0.323]	0.007
31.2	1.614 [0.964–2.379]	0.077 [0.053–0.146]	0.007
62.5	1.631[1.123–2.511]	0.085[0.049–0.253]	0.007
125	0.709 [0.518–1.969]	0.058 [0.047–0.386]	0.007
250	1.644 [1.274–1.664]	0.052 [0.049–0.066]	0.007
500	1.688[0.843–2.479]	0.053 [0.050–0.063]	0.007
1000	1.579 [1.462–2.462]	0.055 [0.053–0.086]	0.007

*Interquartile range (p25– p75)

**Wilcoxon test for paired samples

Table 4 Evaluation of the removal activity in *C. albicans*, *C. parapsilosis*, and *C. tropicalis* specimens in relation to the concentration of *E. oleracea* Mart extract

Concentration (µg/mL)	Removal activity		
	C. albicans	*C. parapsilosis*	*C. tropicalis*
	Mean ± SD	Mean ± SD	Mean ± SD
7.8	0.607 ± 0.096	0.154 ± 0.021	0.440 ± 0.204
15.6	0.504 ± 0.064	0.373 ± 0.213	0.665 ± 0.282
31.2	0.656 ± 0.058	0.196 ± 0.007	0.658 ± 0.168
62.5	0.844 ± 0.161	0.344 ± 0.254	0.805 ± 0.101
125	0.715 ± 0.140	0.497 ± 0.073	0.589 ± 0.065
250	0.962 ± 0.549	0.471 ± 0.060	0.807 ± 0.110
500	0.251 ± 0.112	0.660 ± 0.074	0.648 ± 0.068
1000	1.275 ± 0.279	1.083 ± 0.005	1.640 ± 0.093
p value*	0.016	0.010	0.034

*Kruskal–Wallis test

Since the treatment with *E. oleracea* Mart. extract decreased *Candida* spp. biomass formation, we further tested different concentrations of the extract. Table 3 displays significant differences in medians from before and after the addition of the extract at all concentrations ($p < 0.05$). Therefore, regardless of concentration, antibiofilm activity of açaí berry extract was maintained. Finally, when the removal activity of *E. oleracea* Mart. extract at the different concentrations was analyzed, there was a statistically significant difference between the values obtained for *C. albicans*, *C. parapsilosis*, and *C. tropicalis* (Table 4).

These findings corroborate with those obtained for the antibiofilm activity of *E. oleracea* Mart. extract on glass surface, which was found to prevent biofilm formation. Isolated cells (4 to 6 cells isolated per field) and 99% absence of biofilm (Fig. 2a and b) and 39 isolated cells and absence of biofilm at concentrations of 250, 500, and 1000 µg/mL (Fig. 2c) were observed, preventing biofilm development on glass coverslips. At concentrations of 31.2 to 125 µg/mL, there was also no biofilm formation; however, the presence of aggregative arrangements was observed (Fig. 2j–u). Notably, concentrations 7.8 (a–c*) and 15.6 (v–z) µg/mL were not able to prevent biofilm formation.

Discussion

In the current study, the ability of *C. albicans*, *C. parapsilosis*, and *C. tropicalis* to form biofilms on abiotic surfaces was demonstrated, and the biomass produced varied according to each species. *C. tropicalis* adhered more easily to the abiotic material and was thus associated with greater biofilm formation.

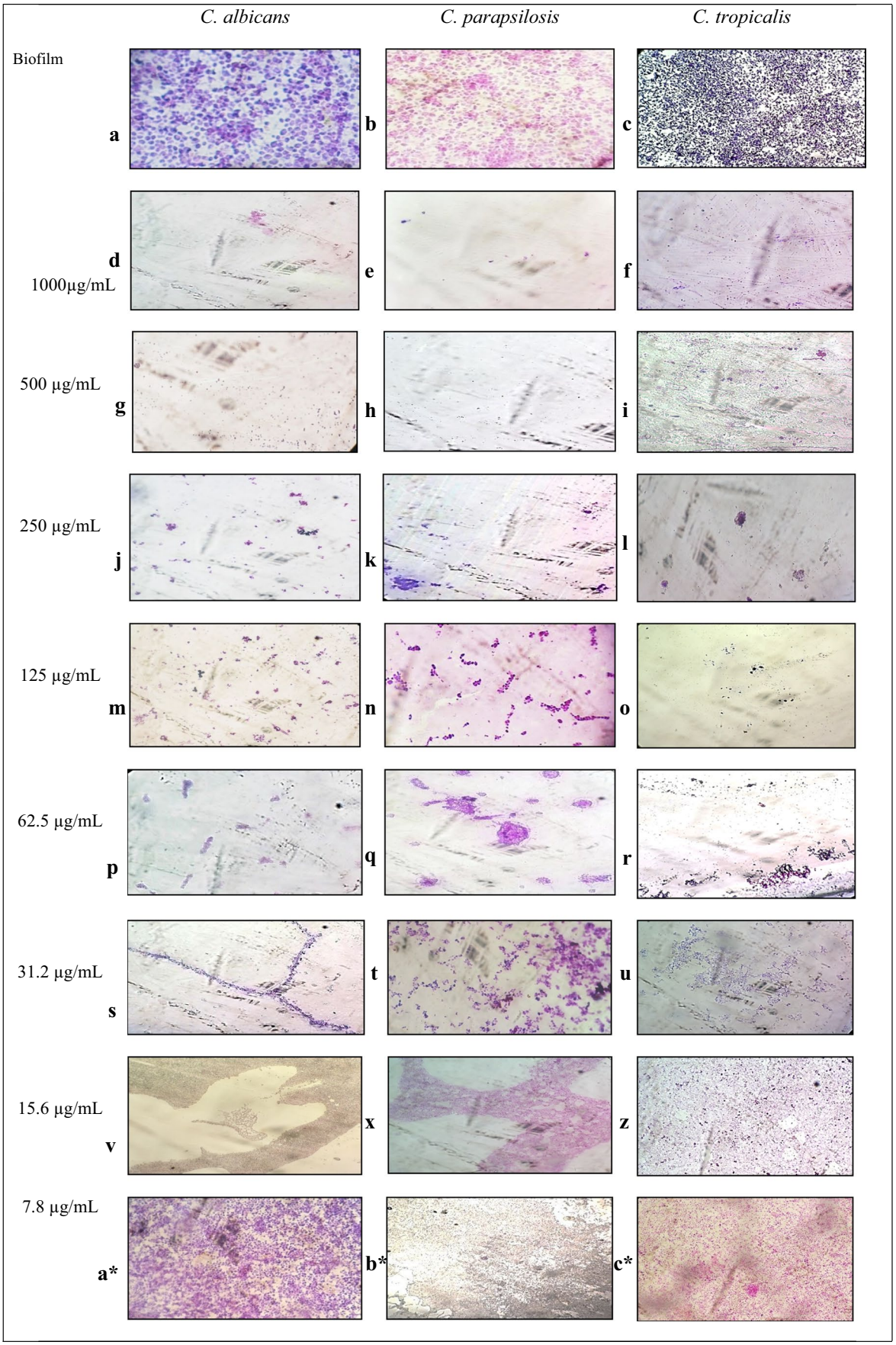
The increasing incidence of drug-resistant pathogens and the toxicity of existing antifungal compounds have increased the interest from antifungal properties of natural products [26]. Several studies have been conducted using natural products to evaluate interference in *C. albicans* biofilm and anticandidal activity on planktonic and biofilm cultures of the *C. parapsilosis* complex [27, 28].

Most of the available antifungals are either ineffective against *Candida* biofilms or exhibit activity at very high concentrations [29, 30]. Plants are rich sources of bioactive molecules exhibiting various biological and pharmaceutical properties. Various phytochemicals are known to possess strong antimicrobial/antifungal activities [31]. Use of these phytochemicals against biofilms could be an excellent strategy [32, 33].

Cannas et al. (2014) evidenced a considerable activity of essential oil of *Myrtus communis* L. against *C. albic*ans and *C. parapsilosis* after 24–48 h [34].

Borges et al. [35] demonstrated an increased adhesion of *C. parapsilosis*, which formed biofilm in copper fragments after 6 and 24 h of incubation, corroborating to our findings. In addition, açaí berry extract, in contact with abiotic surfaces containing biofilms formed by *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, presented with a biofilm removal effect, whereby medians from before and after the treatment with the extract varied significantly in this study.

Several plant extracts, essential oils, and phytomolecules have been found to inhibit biofilm formation by *Candida* spp. [36]. Nair et al. [37] analyzed several phytochemicals and identified plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), a phytochemical of Plumbago species, as a potent antifungal agent against *C. albicans*, with a low minimum inhibitory concentration that was effective at preventing and dispersing biofilms in catheters formed by *C. albicans*. Therefore, in vivo and in vivo evaluation as well as clinical


C. albicans
C. parapsilosis
C. tropicalis
Biofilm
a
b
c
d
1000µg/mL
e
f
500 µg/mL
g
h
i
250 µg/mL
j
k
l
125 µg/mL
m
n
o
62.5 µg/mL
p
q
r
31.2 µg/mL
s
t
u
15.6 µg/mL
v
x
z
7.8 µg/mL
a*
b*
c*

◂**Fig. 2** Antibiofilm effect of different concentration of the *Euterpe oleracea* ethanolic extract (µg/mL) on biofilm of *C. albicans*, *C. parapsilosis*, and *C. tropicalis*

trials is required to further investigate the use of phytochemicals as candidate molecules for anti-biofilm drugs.

Polyphenols and flavonoids are the main chemical compounds from açaí. Polyphenols display excellent biofilm inhibitory activities in *C. albicans*. Studies showed that curcumin, pyrogallol and pyrocatechol possess anti-*Candida* biofilm activity [38]. Epigallocatechin-3-gallate extracted from green tea prevented biofilm formation by *C. albicans* [39].

Epigallocatechin-3-gallate (ECGC) has antifungal activity against human-pathogenic yeasts like *Candida albicans*. Although the mechanistic effects of EGCG are not fully understood, there are results indicating that EGCG binds to lipid membranes and affects the folic acid metabolism of bacteria and fungi by inhibiting the cytoplasmic enzyme dihydrofolate reductase [40].

In the current study, data from before and after the addition of açaí berry extract on biofilms of *C. albicans*, *C. parapsilosis*, and *C. tropicalis* on abiotic surfaces demonstrated a significant difference in biofilm formation at both the lowest (7.8 µg/mL) and the highest (1000 µg/mL) concentrations of the extract. It is worth mentioning that anti-biofilm activity of *E. oleracea* Mart. extract was maintained at all concentrations tested, suggesting that even at low doses, açaí berry extract shows an antifungal effect against *Candida* spp. biofilm. Nadaf et al. [41] observed that *Hymenocallis littoralis* leaf extract at concentrations of up to 70 µg/mL presented with anti-biofilm properties, reducing biomass production by *C. albicans* through interaction with active site residues of adhesin proteins.

The pathogenicity of *Candida* species through various virulence factors, such as adhesion to host surfaces, formation of biofilms and secretion of hydrolytic enzymes has been demonstrated [10]. The apparent increase in the emergence of *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* species can be attributed to better identification methods and has also been associated with clinical impairment, interventions performed, and pharmacological therapy. Although studies to identify virulence factors, particularly in *C. albicans*, are frequent, relatively little is known regarding non-albicans *Candida* species. Millot et al. [42] analyzed several lichen extracts towards identifying their potential activity against *C. albicans* biofilm, eleven of which were found to inhibit biofilm maturation by *C. albicans*.

In relation to the dosage of *E. oleracea* Mart. extract utilized, the greatest inhibitory action on biofilm formation in the species analyzed in this study was obtained at a concentration of 250 µg/mL. Dias-Souza et al. [43] evaluated the effects of different doses of *E. oleracea* Mart. against *Staphylococcus aureus* biofilm and found a minimum biofilm eradication concentration of 250 µg/mL. This indicates that low concentrations are required to obtain an anti-biofilm activity of açaí berry extract against *Candida* spp. biomass production.

Conclusion

In summary, an extract obtained from *E. oleracea* Mart. presented with both anti-biofilm activity and removal effect against *Candida albicans*, *C. parapsilosis*, and *C. tropicalis* biofilms, even when low concentrations were used. These results are important for the development of a new antifungal from a natural product. Further in vitro investigation is required to determine which compounds from açaí berry extract are responsible for the actions observed in the present study before developing in vivo analysis and clinical trials in this regard.

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Data Availability All data is included in the manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Krcmery V, Barnes AJ (2002) Non-albicans Candida spp causing fungaemia: pathogenicity and antifungal resistance. J Hosp Infect 50(4):243–260
2. Arendrup MC (2013) *Candida* and Candidaemia: susceptibility and epidemiology. Dan Med J 60(11):1–32
3. Guinea J (2014) Global trends in the distribution of candida species candidemia. Clinical Microbiology and infection. Clin Microbiol Infect 6:5–10
4. Tamura NK, Fernanda M, Negri N (2007) Fatores de virulência de Candida spp. isoladas de cateteres venosos e mãos de servidores hospitalares. Revista da Sociedade Brasileira de Medicina Tropical 40(1):91–93
5. Lu YSUC, Wang A, Liu H (2017) Hyphal development in Candida albicans requires two temporally linked changes in promoter chromatin for initiation and maintenance. PLoS Biol 7:e1001105
6. Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284(5418):1318–1322
7. Panizo MM, Revia Kina V, Dolande M, Selgrad S (2009) Candida spp. in vitro susceptibility profile to four antifungal agents. Resistance surveillance study in Venezuelan strains. Med Mycol 2:137–43
8. Gulati M, Nobile CJ (2016) Candida albicans biofilms: development, regulation, and molecular mechanisms. Microbes Infect 18(5):310–321

9. Araújo D, Henriques M, Silva S (2017) Portrait of Candida species biofilm regulatory network genes. Trends Microbiol 1:62–75
10. Silva S, Negri M et al (2012) Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemilogy, phatogenecity and antifungal resistence. FEMS Microbiol Rev 2:288–305
11 Cl Seabra, Cm Botelho, Henriques M, Oliveira R (2013) Differential adherence and expression of virulence traits by Candida albicans and Candida parapsilosis in mono- and dual-species cultures in artificial saliva. Mycopathologia 176(1–2):33–40
12. Treviño-Rangel RJ, Rodriguez-Sánchez IPR et al (2015) Biofilm formation and genetic variability of BCR1 gene in the Candida parapsilosis complex. Rev Iberoam Micol 3:180–184
13 Goel S, Mittal S, Chaudhary U (2016) Role of non Albicans Candida Spp. and biofilm in neonatal ICU. Infect Disord Drug Targets 3:192–198
14. Raut JS, Shinde RB, Chauhan NM, Karuppayil SM (2013) Terpenoids of plant origin inhibit morphogenesis, adhesion, and biofilm formation by *Candida* albicans. Biofouling 29(1):87–96
15. Zacchino SA, Butassi E, Cordisco E, Svetaz LA (2017) Hybrid combinations containing natural products and antimicrobial drugs that interfere with bacterial and fungal biofilms. Phytomedicine 37:14–26
16. Sardi JC, Freires IA et al (2017) Unexplored endemic fruit species from Brazil: antibiofilm properties, insights into mode of action, and systemic toxicity of four Eugenia spp. Microb Pathog 105:280–287
17. Al-Sokari SS, El Sheikha AF (2015) In vitro antimicrobial activity of crude extracts of some medicinal plants from Al-Baha Region in Saudi Arabia. J Food Nutr Scie 3(1–2):74–78
18. Khan MSA, Ahmad I (2012) Biofilm inhibition by Cymbopogon citratus and Syzygium aromaticum essential oils in the strains of Candida albicans. J Ethnopharmacol 140(2):416–423
19. Yamaguchi KK, Pereira LF, Lamarão CV, Lima ES, da Veiga-Junior VF (2015) Amazon acai: chemistry and biological activities: a review. Food Chem 179:137–151
20. de Moura RS, Pires KM, Santos Ferreira T, Lopes AA, Nesi RT, Resende AC et al (2011) Addition of acai (Euterpe oleracea) to cigarettes has a protective effect against emphysema in mice. Food Chem Toxicol 49(4):855–863
21. Shin JH, Kee SJ, Shin MG, Kim SH, Shin DH, Lee SK et al (2002) Biofilm production by isolates of Candida species recovered from nonneutropenic patients: comparison of bloodstream isolates with isolates from other sources. J Clin Microbiol 40(4):1244–1248
22. CLSI (2008) Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard, 3rd edn. Document M27-AL3, Wayne, PA. CLSI, Pennsylvania, Clinical and Laboratory Standard Institute
23. Biasoli MS, Tosello ME, Magaró HM (2002) Adherence of Candida strains isolated from the human gastrointestinal tract. Mycoses 45(11–12):465–469
24. Ben Abdeljelil J, Saghrouni F, Emira N, Valentin-Gomez E, Chatti N, Boukadida J, Ben Saïd M, Del Castillo AL (2011) Molecular typing of Candida albicans isolates from patients and health care workers in a neonatal intensive care unit. J Appl Microbiol 111(5):1235–1249
25. Menezes EA, VasconcelosJúnior AA, Ângelo MR, Cunha Mda C, Cunha FA (2013) Correlation between microdilution, Etest, and disk diffusion methods for antifungal susceptibility testing of fluconazole against Candida sp. blood isolates. Rev Soc Bras Med Trop 46(1):106–7
26. de Oliveira LF, Jorge AO, Dos Santos SS (2006) In vitro minocycline activity on superinfecting microorganisms isolated from chronic periodontitis patients. Braz Oral Res 20:202–206
27. Furletti VF, Teixeira IP, Obando-Pereda G, Mardegan RC, Sartoratto A, Figueira GM, Duarte RM, Rehder VL, Duarte MC, Hofling JF (2011) Action of coriandrum sativum L. essential oil upon oral Can-dida albicans biofilm formation. Evid Based Complement Altern Med 2011:985832
28. Pires RH, Montanari LB, Martins CH, Zaia JE, Almeida AM, Matsumoto MT, Mendes-Giannini MJ (2011) Anticandidal efficacy of cinnamon oil against planktonic and biofilm cultures of Candida parapsilosis and Candida orthopsilosis. Mycopathologia 172:453–464
29. Shinde RB, Raut JS, Karuppayil MS (2012) Biofilm formation by Can- dida albicans on various prosthetic materials and its fluconazole sensitivity: a kinetic study. Mycoscience 53(3):220–226
30. Mukherjee PK, Chandra J, Kuhn DM, Ghannoum MA (2003) Mechanism of fluconazole resistance in Candida albicans biofilms: phase-specific role of efflux pumps and membrane sterols. Inf Immun 71(8):4333–4340
31. Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12(4):564–582
32. Bink A, Pellens K, Cammue BP, Thevissen K (2011) Anti-biofilm strategies: how to eradicate Candida biofilms? Open Mycol J5:29–38
33. Raut JS, Shinde RB, Chauhan NM, Mohan KS (2013) Terpenoids of plant origin inhibit morphogenesis, adhesion, and biofilm formation by Candida albicans. Biofouling 29(1):87–96
34. Cannas S, Molicotti P, Usai D, Maxia A, Zanetti S (2014) Antifungal, anti-biofilm and adhesion activity of the essential oil of Myrtus communis L. against Candida species. Nat Prod Res 28(23):2173–7
35. Borges KRA, Pimentel IV, Lucena LCLDS, Silva MACND, Monteiro SG, Monteiro CA et al (2018) Adhesion and biofilm formation of *Candida* parapsilosis isolated from vaginal secretions to copper intrauterine devices. Rev Inst Med Trop Sao Paulo 22(60):e59
36. Raut JS, Karuppayil SM (2016) Phytochemicals as Inhibitors of Candida Biofilm. Curr Pharm Des 27:1–24
37. Nair SV, Baranwal G, Chatterjee M, Sachu A, Vasudevan AK, Bose C et al (2016) Antimicrobial activity of plumbagin, a naturally occurring naphthoquinone from Plumbago rosea, against Staphylococcus aureus and Candida albicans. Int J Med Microbiol 306(4):237–248
38. Shahzad M, Sherry L, Rajendran R, Edwards CA, Combet E, Ramage G (2014) Utilising polyphenols for the clinical management of Can- dida albicans biofilms. Int J Antimicrob Agents 44(3):269–73
39. Evensen NA, Braun PC (2009) The effects of tea polyphenols on Candida albicans: inhibition of biofilm formation and proteasome inactivation. Can J Microbiol 55(9):1033–1039
40. Steinmann J, Buer J, Pietschmann T, Steinmann E (2013) Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. Br J Pharmacol 168(5):1059–1073
41. Nadaf NH, Parulekar RS, Patil RS, Gade TK, Momin AA, Waghmare SR et al (2018) Biofilm inhibition mechanism from extract of Hymenocallis littoralis leaves. J Ethnopharmacol 10(222):121–132
42 Millot M, Girardot M, Dutreix L, Mambu L, Imbert C (2017) Antifungal and anti-biofilm activities of acetone lichen extracts against Candida albicans. Molecules 22(4):pii: E651
43. Dias-Souza MV, Dos Santos RM, Cerávolo IP, Cosenza G, Ferreira Marçal PH, Figueiredo FJB (2018) Euterpe oleracea pulp extract: chemical analyses, antibiofilm activity against Staphylococcus aureus, cytotoxicity and interference on the activity of antimicrobial drugs. Microb Pathog 114:29–35