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RESEARCH ARTICLE OPEN ACCESS

EFFECT OF *CNIDOSCOLUS URENS (L.)* ARTHUR METHANOLIC EXTRACT ON VIRULENCE ATTRIBUTES OF *CANDIDA ALBICANS* SENSU STRICTO

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ABSTRACT

Candida albicans is one of the most prevalent pathogenic fungi worldwide and in recent decades has shown resistance to azoles and echinocandins. Therefore, the search for therapeutic alternatives is necessary in order to combat infections caused by this fungus. This study evaluated the chemical composition and the effects of *Cnidoscolus urens* methanolic extract, derived from the plant latex, on virulence attributes of *C. albicans*. The ability of the methanolic extract of *C. urens* to inhibit growth, interaction with abiotic substrates, biofilm formation and interaction with the RAW 264.7 cell line was investigated. The analysis of *C. urens* methanolic extract suggested the presence of quinic acid as a major latex component. Results of *in vitro* assays demonstrates that *C. urens* methanolic extract significantly inhibited growth, adhesion to the glass abiotic substrate, biofilm formation (pre-treatment stage) and interaction with the RAW 264.7 cell line. These results demonstrated that *C. urens* methanolic extract inhibited aspects associated with the virulence of *C. albicans*. These effects may be associated with quinic acid, a major component of *C. urens* latex, which indicates that quinic acid may be a promising new therapeutic agent in the treatment of candidiasis.

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INTRODUCTION

In the last decades, infections caused by fungi of *Candida* genus have increased considerably (Ghazi *et al*., 2019; Braga *et al*., 2018; Matta *et al*., 2017). These microorganisms can cause from superficial infections to nosocomial invasive and hematogenic infections, mainly in individuals hospitalized for prolonged periods, in intensive care units, under the use of corticosteroids, antibiotics, parenteral nutrition and submitted to invasive medical procedures (Pappas *et al*., 2018; Kullberg *et al*., 2015; Yapar *et al*., 2014). *Candida albicans* is the most predominant species of *Candida* genus and is associated with most cases of candidemia and invasive infections in patients worldwide (Nucci *et al*., 2013; Papon *et al*., 2013; Pfaller *et al*., 2014a; Pfaller *et al*., 2014b; Guinea *et al*., 2014; Braga *et al*., 2018; Ghazi *et al*., 2019; Adam *et al*., 2021; Kajihara *et al*., 2022). *C. albicans* can cause infections ranging from vaginal to invasive infections in hospitalized and immunocompromised patients (Poulain, 2015). Some virulence factors such as the production of hydrolytic enzymes (proteases, phospholipases and lipases), transition from yeast to hyphae, expression of surface adhesins, biofilm formation, allow the establishment of infection in a susceptible host (Dadar *et al*., 2018; Kadosh *et al*., 2019). These fungi have been showing resistance to several antifungals used in medical practice and for this reason are associated with high mortality rates among infected individuals (Kullberg *et al*., 2015; Juang, 2007). Therefore, studies that develop new drugs for the treatment of infections caused by these fungi are extremely necessary. Knowledge from traditional medicine has brought alternatives, such as the use of plants, for the treatment of

infections caused by different microorganisms (Agra *et al*., 2007, 2008; Petrovska, 2012). Some studies have shown the importance of plants and highlighted fundamental knowledge that can be used for the development of effective new drugs for the treatment of different diseases (Albuquerque *et al*., 2007; Ferreira *et al*., 2011, Nunes *et al*., 2016). The *Cnidoscolus* Pohl genus is native to Brazil and has approximately 50-70 species distributed in the Brazilian Northeast (Webster, 1994). One of the main characteristics of this genus is the presence of stinging trichomes in almost all vegetative and floral parts of the plant which, when touched, they can generate different symptoms such as hives, severe localized pain and in some cases fainting (Muenscher, 1958; Melo and Sales 2008). *Cnidoscolus urens* (L.) Arthur popularly known as urtiga or cansanção is a plant found in the Brazilian Caatinga Biome. In traditional medicine, stem bark is used to treat cancer, hemorrhage (action on blood clotting), inflammation, pain and latex is used to treat external ulcers. (Albuquerque *et al*., 2006; Albuquerque *et al*., 2007; Agra *et al*., 2007; Melo *et al*., 2011). Although there are few studies with *C. urens*, some biological activities have already been evidenced. Menezes *et al*. (2014) showed the presence of cysteine proteases, in a protein rich-fraction from *C. urens* leaves. Cysteine protease are active at acidic pH and under high temperatures, exhibiting increased activity in the presence of reducing agents. In addition, fibrinogenolytic, procoagulant and fibrinolytic activity was observed in the F1.0 protein-rich fraction of *C.urens*, indicating a possible application of these proteases as anti-hemorrhagic, thrombolytic and wound healing agents (Menezes *et al*., 2014). Due to the biological potential that *C. urens* presents as well as its use in traditional medicine, this study aimed to investigate the effects of *C. urens* methanolic extract, derived from the plant latex, on virulence attributes of *C. albicans*. We verified that the methanolic extract of *C. urens* was able to inhibit some virulence factors of *C. albicans*.

MATERIALS AND METHODS

Plant material: The latex from of *C. urens* stem was collected in the city of Floriano at *Campus* Amilcar Ferreira Sobral (Federal University of Piauí- UFPI) (latitude: 6°47'21", longitude: 43°02'24") in the state of Piauí, Brazil. Botanical identification was carried out by Prof. Dr. André Laurênio de Melo from the Rural University of Pernambuco, Academic Unit of Serra Talhada, Serra Talhada, Pernambuco, Brazil. A voucher specimen was deposited at the Herbarium Graziela Barroso of the Federal University of Piauí (UFPI) with number: TEPB 31,225 – Euphorbiaceae *Cnidoscolus urens* (L.) Arthur. The latex was collected from the stem of *C. urens* and dried in an oven at 60 °C for 12h. Then, the dried latex (100 mg) was incubated with 1 ml of methanol for extraction of chemicals. The resulting mixture was vortexed and centrifuged for 10 minutes at 3000 rpm. The supernatant was collected and used in all experiments (Ishnava *et al*., 2012).

Microorganism and cultivation: *Candida albicans* (ATCC 10231) were grown in Sabouraud medium for 48 h at 37 °C without shaking. Fungal growth was measured by counting yeasts in a Neubauer chamber (Abi-chacra *et al*., 2013).

Cells: The RAW 264.7 cell line (TIB-71 code) from BALB/c mice (macrophages) was obtained from the Cell Bank of the Federal University of Rio de Janeiro (UFRJ). Animal cells were maintained and grown to confluence in 25 cm^2 culture flasks containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10.000 IU penicillin and 1.000 IU streptomycin at 37°C/5% CO_2. The pH was maintained at 7.2 by adding 3 g/l of HEPES and 0.2 g/l of $NaHCO_3$ to the culture medium. The initial inoculum was 5 x 10^4 cells/ml, being subcultured every 2 days, and maintained in logarithmic phase of growth (Gandra *et al*., 2019).

Chemical profile by electrospray ionization-mass spectrometry: The latex extract dissolved in methanol (HPLC/Spectro Tedia) at a concentration of 100-110 ng/µL were analyzed by direct infusion on a MicrOTOF Q-II mass spectrometer (Bruker Daltonics) equipped with an electrospray ionization source (ESI). The injection flow was 180 µL/h and parameters of the ESI source were: capillary voltage: 3.5 kV; pressure of the nitrogen nebulizer: 2 bar; flow of drying gas: 9 L/min, and drying gas temperature: 190 °C. The mass spectrometer was calibrated with sodium formate solution and the mass spectra were acquired in the negative (−) ionization mode with full mass range of *m/z* 100-1000. Data processing was performed using *Compass* 1.3 data analysis software (version 4.0, Bruker Daltonics).

Effect of C. urens methanolic extract on the fungal growth: Yeasts (10^6 cells/ml) were treated with *C. urens* methanolic extract at 10, 25, 50 and 100 µg/ml concentrations for 18 h and 24 h. A control group (cells without treatment) was also prepared. After the treatment period, the yeasts were centrifuged (4000 g/ 10 min/ 4 °C) and washed three times with PBS (phosphate saline buffer; pH 7.2). Yeast growth was measured by counting in a Neubauer chamber. Then, the cell suspension was diluted to obtain 1x10^4 cells/ml. The same dilution was performed in all systems and 10 µl of the cell suspension was transferred to the solid Sabouraud Dextrose Agar medium. After 48h of incubation at 37 °C, the colony forming units (CFU) were counted and the growth of treated and untreated systems was evidenced. (Brajtburg *et al*., 1981). The IC_{50} (50% inhibitory concentration) was measured through linear regression using the log of the number of viable cells versus the concentration of the methanolic extract used. The linear regression analysis was performed using the BioEstat 5.3 program (Instituto Mamirauá, Brazil).

Effect of C. urens methanolic extract on adhesion to inert surfaces: Fungi (10^6 cells) were pre-treated with *C. urens* methanolic extract at 10, 25, 50 and 100 µg/ml concentrations for 24 h at 37 °C. Then, the yeasts were subjected to interaction with glass coverslips, previously washed with Extran for 2 h, incubated with 70% ethanol for 30 minutes and sterilized in an oven at 180 °C for 2 h and with polystyrene plates (24 wells) for 2 h at 37 °C. After this period, the glass coverslips and the polystyrene plate wells were washed three times with PBS to remove non-adherent cells and fixed with methanol for 30 minutes for counting in optical (Olympus) and inverted microscope (Olympus). To determine adherence to abiotic surfaces, a total of five fields per coverslip (three coverslips / wells for each strain) were analysed and the average of each coverslip or well determined. The experiments were carried out in triplicate (Abi-chacra *et al*., 2013).

Effect of C. urens methanolic extract on biofilm formation: Fungal cells (10^6) pre-treated with *C. urens* methanolic extract (10, 25, 50 and 100 µg/ml) for 24 h were transferred to a 96-well polystyrene microtiter plate and incubated for additional 48 h at 37 °C to form the mature biofilm (pre-treatment stage). In addition, fungi (10^6 cells) were incubated with *C. urens* methanolic extract (10, 25, 50 and 100 µg/ml) during the 48 h of biofilm formation in the polystyrene plate (during stage) and finally yeasts (10^6 cells) were transferred to the polystyrene plate and incubated for 48 h to form the mature biofilm and after the system was treated with *C. urens* methanolic extract (10, 25, 50 and 100 µg/ml) for an additional 24 h (post-treatment stage). Then, the wells were washed three times with PBS to remove non-adherent cells. The biomass of biofilm was evidenced by the crystal violet assay. The wells were incubated with 0.4% violet crystal at room temperature for 5 min. Then, the systems were washed three times with distilled water to remove excess stain and absolute ethanol was added for 5 min. The solution was transferred to another 96-well plate and the absorbance was measured at 550 nm in an ELISA reader (EZ Read 400, Biochrom Ltd, Cambridge, England) (RAVI *et al*., 2009). Additionally, the biofilm was grown on glass slides (1cm x 1cm) that were gently added to the wells of a 24-well plate in the presence or absence of *C. urens* methanolic extract (10, 25, 50 and 100 µg/ml) for 48h. After the incubation period, the slides were washed with sterile distilled water to remove non-adherent cells and stained with 0.4% crystal violet for 5 minutes. The slides were then washed again with sterile distilled water and air dried. The biofilm formed was observed under an optical microscope (Olympus) at 400x magnification (Muthamil *et al*., 2018).

Effect of C. urens methanolic extract on macrophage toxicity: The cytotoxicity *C. urens* methanolic extract on murine macrophages was evaluated in the RAW 264.7 cell line using the MTT (3-[4,5-dimethylthiazol-zyl]-2,5-diphenyltetrazolium bromide) assay. In a 96-well plate, 100 µL of DMEM medium supplemented (fetal bovine serum (10%), 10.000 IU penicillin and 1000 IU streptomycin) was added and then 2 x 10^5 macrophages per well. These cells were incubated at 37°C/5% CO_2 for 4 h for cell adhesion. After that time, two washes were performed with DMEM medium supplemented (fetal bovine serum (10%), 10.000 IU penicillin and 1000 IU streptomycin) to remove the cells that did not adhere. Subsequently, 100 µL of DMEM supplemented (fetal bovine serum (10%), 10.000 IU penicillin and 1000 IU streptomycin) were added together with the diluted methanolic extract (125:0, 100:25, 75:50, 50:75, 25:100, and 0:125 µg/ mL), serially, at concentrations from 800 to 6.25 µg/mL. Then the macrophages were incubated for 48h and, at the end of the incubation, 10 µL of MTT diluted in DMEM medium at 5 mg/mL was added. Afterwards, the cells were incubated for another 4h at 37ºC/5% CO_2, and then the supernatant was discarded for the addition of 100 µL of DMSO in all wells. Then, the plate was placed under agitation for about 30 min in a Kline shaker (model AK 0506) at room temperature for complete dissolution of formazan. Finally, the plate was read at 550 nm in an ELISA reader (ELx800). The results were expressed in percentage and in mean cytotoxic concentration (CC50) and the control group was considered to have 100% cell viability (Oliveira *et al*., 2017).

Effect of C. urens methanolic extract on fungal-macrophage interaction: In the interaction assay, untreated and treated fungi (10µg/mL, 25µg/mL, 50µg/mL and 100µg/mL) were added to the monolayer of RAW 264.7 cells with the methanolic extract of *C. urens* in a proportion of 10:1 (fungi: macrophages), and the systems were incubated at 37 °C in 5% CO_2 for 2 hours in 24-well plates. The systems were washed with PBS and stained with the Panótico kit. In each experiment, 300 animal cells were randomly analyzed on each of three triplicate slides. An optical microscope immersion objective (Olympus) was used for the analysis of the experiments (Gandra *et al*., 2019).

Statistical analysis

All experiments were performed at least three times, using triplicates. The results were analyzed statistically by the one-way ANOVA test, the Dunnett test and Bonferroni's test using the BioEstat 5.0 program (Instituto Mamirauá, Brazil). P values less than or equal to 0.05 (*p<0.05) were considered statistically significant.

RESULTS

Chemical characterization of methanolic extract of latex from C. urens stem: The analysis of *C. urens* methanolic extract by electrospray ionization coupled to time-of-flight mass spectrometry (ESI-TOFMS) suggested the presence of quinic acid and derivatives (Figure 1). Shikimic acid [M−H]: 173.05, caffeic acid [M−H]: 179.06, and dihydroferulic acid [M−H]: 195.05 were the single acids. In addition, the abundant peak with [M−H]: 191.05 was identified as quinic acid. Fatty acids were identified based on [M−H]: 281.17 (7), 297.15 (8), 311.17 (9), 321.09 (10), and 325.23 (11). The [M−H] at 255.23 and 269.24 suggests the structures of deoxofaveline and a methyl derivative of deoxofaveline. Quinic acid derivatives were represented by [M−H]: 353.20 (12), 515.16 (13), and 533.17 (14) that were identified as chlorogenic acid, dicaffeoylquinic acid, and a methyl derivative from dicaffeoylquinic acid, respectively.

Effect of *C. urens* methanolic extract on the growth of *C. albicans:* The effect of *C. urens* methanolic extract on the growth of *C. albicans* was evaluated by counting colony-forming units (CFU). The *C. urens* methanolic extract was able to inhibit the growth of *C. albicans* (p <0.0001 and p= 0.0188) after 18 and 24 h of treatment at the highest concentration tested (100 µg/ml) (Figure 2).

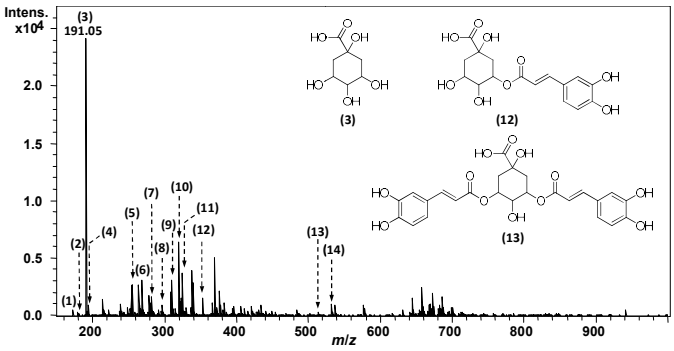

Figure 1. ESI(−)-MS of methanolic extract from latex of *C. urens* stem. Chromatogram obtained by ESI-TOFMS indicating the presence of quinic acid and derivatives. Quinic acid is the major component of *C. urens* latex. (1) shikimic acid, (2) caffeic acid, (3) quinic acid, (4) dihydroferulic acid, (5) deoxofaveline, (6) deoxofaveline derivative, (7) octadecenoic acid, (8) nonadecanoic acid, (9) eicosanoic acid, (10) heneicosadienoic acid, (11) heneicosanoic acid, (12) chlorogenic acid, (13) dicaffeoylquinic acid, (14) dicaffeoylquinic acid derivative

The IC_{50} values for *C. albicans* were 46,7 µg/ml and 40,6 µg/ml (18 and 24 h of treatment) respectively.

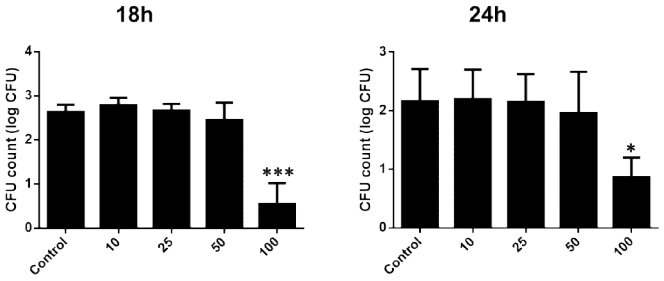

Figure 2. Effect of *C. urens* methanolic extract on the growth of *C. albicans.*

The cells of *C. albicans* were treated for 18 and 24 h with 10, 25, 50 and 100 µg/ml of *C. urens* methanolic extract and the growth was measured by counting the colony-forming units. The values represent the means ± standard deviation of three experiments performed in triplicate. *Significant values in relation to the control (cells without treatment), p <0.05-test one-way ANOVA.

Effect of *C. urens* methanolic extract on the adhesion of *C. albicans* to different abiotic substrates: The inhibition of *C. albicans* cell adhesion to different abiotic substrates by *C. urens* methanolic extract was also investigated. The *C. urens* methanolic extract inhibited the adhesion of *C. albicans* (p= 0.029) to the glass abiotic substrate at the highest concentration tested (100 µg/ml). There was no inhibition of adhesion to the polystyrene substrate (Figure 3).

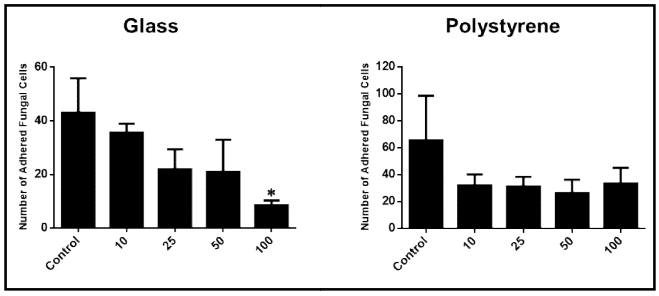

Figure 3. Effect of the *C. urens* methanolic extract on the adhesion of *C. albicans* to different abiotic substrates

The cells of *C. albicans* were treated for 24 h with 10, 25, 50 and 100 µg/ml of *C. urens* methanolic extract. Then, fungi were allowed to interact with both polystyrene and glass surfaces. Cell adhesion was assessed by counting in an optical and inverted microscope. The values represent the means ± standard deviation of three experiments performed in triplicate. *Significant values in relation to the control (cells without treatment), p <0.05-test one-way ANOVA.

Effect of *C. urens*methanolic extract on the biofilm formation of *C. albicans:* In order to evaluate the effects of *C. urens* methanolic extract on the biomass of *C. albicans* biofilm was used the violet crystal assay. The methanolic extract of *C. urens* inhibited the formation of *C. albicans* biofilm in the pre-treatment phase at all concentrations tested (10 µg/ml: p= 0.014; 25 µg/ml: p= 0.031; 50 µg/ml: p= 0.039 and 100 µg/ml: p= 0.019), which can be evidenced by the decrease in its biomass (Figure 4). Furthermore, we can observe the formation of loose microcolonies and individual cells associated with polystyrene (Figure 5).

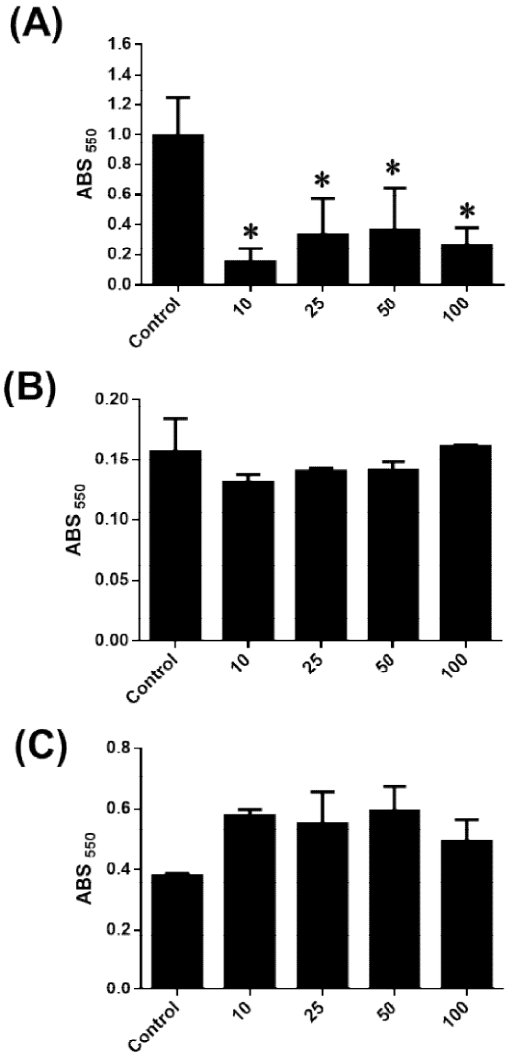

Figure 4. Effect of the *C. urens* methanolic extract on the biofilm formation of *C. albicans*

Fungi (10^6) were pre-treated with *C. urens* methanolic extract for 24 h and then incubated in 96-well polystyrene plates containing Sabouraud medium for 48 h to form a mature biofilm (A). Furthermore, *C. urens* methanolic extract was incubated with the fungi (10^6) during the 48 h of biofilm formation on the polystyrene plate (B). Finally, after the mature biofilm was formed (48 h), the system was treated with *C. urens* methanolic extract for an additional 24 h (C). The biomass of the biofilm was measured by the violet crystal assay at 550 nm. The values represent the means ± standard deviation of three experiments performed in triplicate. *Significant values in relation to the control (cells without treatment), p <0.05-test one-way ANOVA.

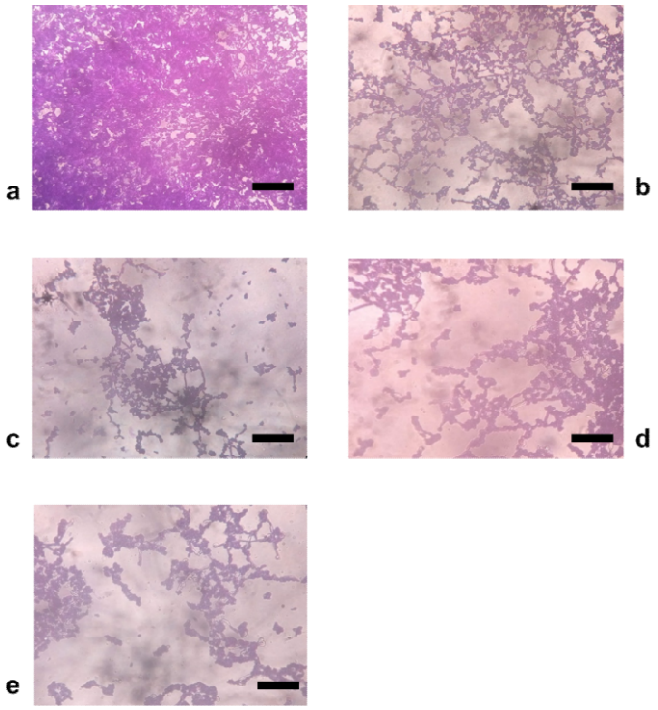

Figure 5. Effect of the *C. urens* methanolic extract on the biofilm formation of *C. albicans*

Light microscopy of the biofilm formed by *C. albicans* treated with *C. urens* methanolic extract (control (a), 10µg/mL (b), 25µg/mL (c), 50µg/mL (d) and 100µg/mL (e)). It is possible to observe the formation of loose microcolonies and individual cells adhered to the polystyrene. Bars, 50 µm.

Effect of *C. urens* methanolic extract on macrophage toxicity: The cytotoxicity of *C. urens* methanolic extract to RAW 264.7 cells were evaluated using the MTT assay. The results showed that *C. urens* methanolic extract was not cytotoxic to murine macrophages at the maximum concentration tested (800 µg/mL) (Figure 6), where the cell viability of macrophages was reduced by only 40%.

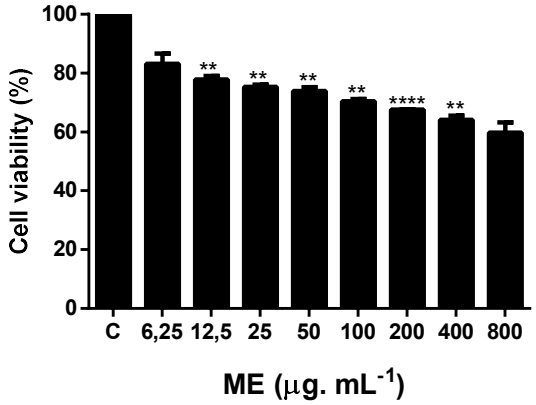

Figure 6. Effect of the *C. urens* methanolic extract on macrophage toxicity

Effect of methanolic extract *C. urens* on the viability of macrophages of the RAW 264.7 strain. *C. urens* methanolic extract was evaluated using the 3-[4,5-dimethylthiazol-zyl]-2,5-diphenyltetrazolium bromide (MTT) test. Values represent the means ± standard deviation of three experiments performed in triplicate. *Significant values compared to control (untreated cells), p<0.05-test one-way ANOVA.

Effect of C. urens methanolic extract on fungal-macrophage interaction: *C. albicans* cells were pre-treated with *C. urens* methanolic extract and later interacted with macrophages of the RAW 264.7 lineage for 2 h. *C. urens* methanolic extract inhibited the cellular interaction of *C. albicans* with murine macrophages at concentrations of 25 (p=0.035), 50 (p=0.038) and 100µg/mL (p=0.003) (Figure 7). During the process of fungal-macrophage cell interaction, no changes were observed in the fungal cell differentiation process (yeast-hypha/pseudohypha) (Figure 8). These results indicate that the pre-treatment of *C. albicans* with *C. urens* methanolic extract impairs the interaction of the fungus with the host cell.

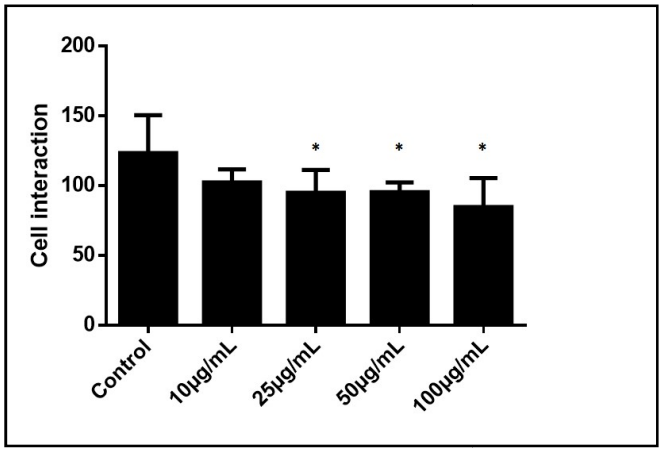

Figure 7. Effect of the *C. urens* methanolic extract on fungal-macrophage interaction

The fungi were pre-treated with *C. urens* methanolic extract at concentrations of 10µg/mL, 25µg/mL, 50µg/mL and 100µg/mL for 24 h. Then, fungi interacted with macrophages for 2 h. Fungal cells incubated in the absence of *C. urens* methanolic extract were used as controls. Interaction was measured by randomly counting at least 300 cells in each triplicate using a light microscope. The values represent the means ± standard deviation corresponding to three experiments performed in triplicate. *Significant values compared to control (untreated cells), p<0.05-test one-way ANOVA.

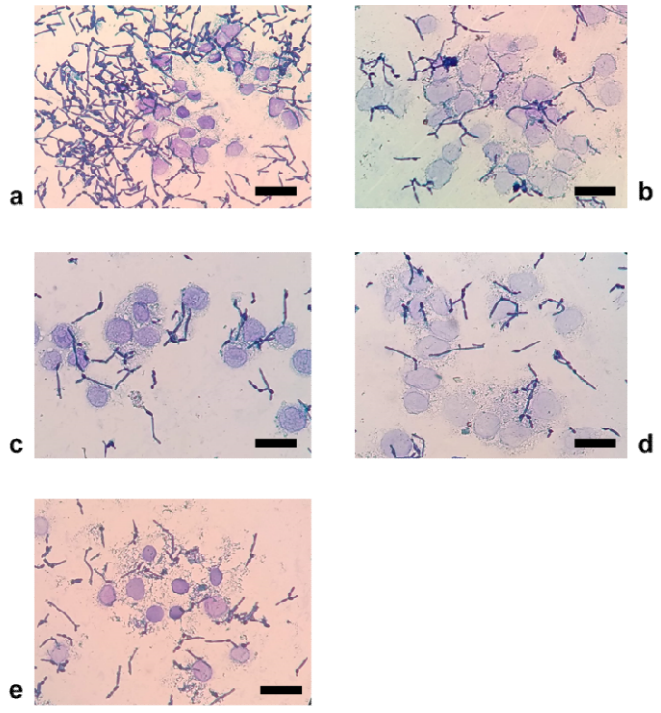

Figure 8. Effect of the *C. urens* methanolic extract on fungal-macrophage interaction.

Light microscopy showing interaction events between untreated (a) and treated (10µg/mL (b), 25µg/mL (c), 50µg/mL (d) and 100µg/mL (e)) with *C. urens* methanolic extract and the RAW 264.7 cell line. After 2 hours of interaction it is possible to observe the process of cell differentiation in *C. albicans*. Bars, 50 µm.

DISCUSSION

Traditional medicine has brought a lot of knowledge about the use of different plants to treat infections caused by microorganisms (Agra *et al*., 2007; Agra *et al*., 2008; Petrovska, 2012). Latex from *C. urens* has been used in Brazilian Northeast to treat external ulcers that can be caused by different microorganisms, including fungi of the *Candida* genus (Galimbert *et al*., 1989; Agra *et al*., 2007; Foroozan *et al*., 2011; Arun *et al*., 2019). This knowledge from traditional medicine encouraged us to investigate the effects of compounds from *C. urens* latex on growth, adhesion, biofilm formation of *C. albicans* and fungal-macrophages interaction. These virulence factors are crucial for the establishment of infections by this microorganism. The effect of *C. urens* methanolic extract on growth was investigated by counting the colony forming units. The results showed that *C. urens* methanolic extract inhibited the growth of *C. albicans* in the highest concentration tested. *Cnidoscolus* spp. already has been reported for its antimicrobial properties against various bacterial pathogens and some fungi (Paredes *et al*., 2016; Ribeiro *et al*., 2017), but not for *Candida* spp. The growth inhibition observed in our study is probably related to quinic acid, the major component of *C. urens* methanolic extract. The effect of quinic acid derived from the leaves of *Syzygium cumini* associated with undecanoic acid was evaluated in a previous study (Muthamil *et al*., 2018), in which no growth inhibition of *C. albicans* was observed. However, in our study, we evaluated the effect of the crude extract of *C. urens* on the growth of species of the genus *Candida*. In the crude methanolic extract, we observed the presence of other compounds in addition to quinic acid, such as caffeic acid, chlorogenic acid, dihydroferulic acid, which can similarly to undecanoic acid (Muthamil *et al*., 2018) might have a synergistic action with quinic acid amplifying its antimicrobial action. Santos *et al*. (2016) showed the antimicrobial potential of chlorogenic acid against *C. albicans*, which may suggest a synergistic action of chlorogenic acid with quinic acid in our study.

The cell adhesion process is crucial for the virulence of *Candida* spp. (Martin *et al*., 2018). The adhesion to abiotic and biotic surfaces favors the development of biofilm, which allows the escape of the fungi from the host's immune response, resistance to different antifungals used in the clinic and to adverse environmental conditions (Krachler and Orth 2013; Modrzewska and Kurnatowski, 2015). Due to the role of cell adhesion in the virulence of *Candida* spp. we investigated the effect of methanolic extract *C. urens* on the cellular adhesion of *C. albicans* to abiotic substrates (glass and polystyrene). The methanolic extract of *C. urens* was able to inhibit the adhesion of *C. albicans* to the glass abiotic substrate at the highest concentration tested. Previous studies have demonstrated the action of quinic acid and shikimic acid on the cell membrane of *S. aureus*. Quinic acid and shikimic acid promote membrane hyperpolarization, loss of membrane integrity, and interact with membrane proteins and lipids (Bai *et al*., 2017). Although the cell membrane of *C. albicans* has a different composition and function than the cell membrane of *S. aureus*, interactions between quinic acid and shikimic acid, found in *C. urens* methanolic extract, may occur with surface components that lead to a decrease in cell adhesion to glass observed in our study. Microbial biofilms represent a major challenge for modern medicine, because they promote the persistence of the fungus in the host and the establishment of a chronic infection in these individuals. (Santos *et al*., 2015; Sardi *et al*., 2014; Desai *et al*., 2014; Mathe *et al*., 2013; Taff *et al*., 2013). Thus, the discovery of new drugs that can inhibit biofilm formation is extremely important and necessary for the treatment of infections caused by fungi. The treatment with the methanol extract *C. urens* led to a considerable reduction in the biomass of the *C. albicans* biofilm in the pretreatment phase at all concentrations tested, as well as the formation of loose microcolonies

and individual cells associated with polystyrene. Muthamil *et al*. (2018) observed a synergistic antibiofilm activity of quinic acid and undecanoic acid against *Candida* spp. In this study, quinic acid and undecanoic acid were able to alter the content of extracellular polymeric matrix such as polysaccharides, amino acids and fatty acids present in the biofilm, leading to an inhibition of the extracellular matrix. The extracellular matrix plays a crucial role in maintaining the biofilm as it allows the evasion of the host immune response and the antifungal agents used to treat these infections (Mello *et al*., 2017). In our study the major component of *C. urens* methanolic extract is quinic acid, however we observed the presence of other chemical constituents such as caffeic acid and chlorogenic acid, that have already been described in the literature as *S. aureus* biofilm inhibitors (Luís *et al*., 2014). Therefore, caffeic acid and chlorogenic acid might have synergistic action with quinic acid and inhibit the formation of biofilm in *C. albicans*.

The evaluation of the *in vitro* toxicity of extracts from plants is extremely important, as it can be used to indicate the possible toxicity effects that may occur *in vivo* (Carballo *et al*., 2002). In the present study, the *in vitro* model used cells of the RAW 264.7 lineage, which are macrophages from a cell line transformed by the Abelson leukemia virus derived from BALB/c mice (Taciak *et al*., 2018). *C. urens* methanolic extract did not show cytotoxicity for RAW 264.7 cells at the maximum concentration of 800 µg/mL according to the cytotoxicity assay. Ribeiro *et al*. (2021) observed a similar effect with seed oil from *C. quercifolius*. Thus, *C. urens* methanolic extract can be used for therapeutic purposes due to its low toxicity. Furthermore, the methanolic extract of *C. urens* inhibited the fungus-macrophage interaction at concentrations of 25, 50 and 100µg/mL, however it did not inhibit the process of cell differentiation in *C. albicans*. Quinic acid is the major component of *C. urens* methanolic extract. Quinic acid is a cyclic polyol that has antioxidant, anti-inflammatory, hepatoprotective, anticancer and antimicrobial properties (Padmini *et al*., 2013; Jang *et al*., 2017; Muthamil *et al*., 2018). Chlorogenic and caffeic acids also have antimicrobial properties, exhibiting activity against hepatitis B virus (HBV) in vitro on HepG2.2.15 cells (Wang *et al*., 2009). Probably the inhibition of the interaction between *C. albicans* and RAW 264.7 cells observed in our study is related to the presence of quinic acid, chlorogenic and caffeic acids present in *C. urens* methanolic extract.

CONCLUSIONS

The present study demonstrates for the first time the chemical profile and the biological properties of the methanolic extract from the latex of *C. urens*. *C. urens* methanolic extract inhibited growth, adhesion to the abiotic glass substrate, fungal-macrophage interactions and the biofilm formation of *C. albicans*, demonstrating its action on several important virulence factors for these fungi. These activities can be attributed to quinic acid, the major component of latex from *C. urens* stem. Quinic acid may be a new effective therapeutic target in the treatment of infections caused by *C. albicans*.

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