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ORIGINAL PAPER

Lectins ConA and ConM extracted from *Canavalia ensiformis* (L.) DC and *Canavalia rosea* (Sw.) DC inhibit planktonic *Candida albicans* and *Candida tropicalis*

Victor Juno Alencar Fonseca[1] · Ana Lays Braga[1] · Ray Silva de Almeida[1] · Taís Gusmão da Silva[1] · Josefa Carolaine Pereira da Silva[1] · Luciene Ferreira de Lima[1] · Maria Helena Cruz dos Santos[2] · Romério Rodrigues dos Santos Silva[2] · Claudener Souza Teixeira[2] · Henrique Douglas Melo Coutinho[1] · Maria Flaviana Bezerra Morais-Braga[1]

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Abstract

Lectins participate in the defense against microorganisms and in signaling the damage caused by pathogens to the cell surface and/or intracellular in plants. This study aims to analyze the antifungal potential of lectins extracted from seeds of *Canavalia ensiformis* (L.) DC and *Canavalia rosea* (Sw.) DC, against *Candida albicans* and *Candida tropicalis*. The antimicrobial tests were performed by microdilution against *Candida* spp. The test to verify the combined lectin/fluconazole effect was performed using subinhibitory concentrations of lectins and with antifungal ranging from 0.5 to 512 µg/mL. The ability to inhibit the morphological transition of *Candida* spp. was evaluated by microcultivation in a moist chamber. The results of the minimum inhibitory concentration revealed no antifungal activity against the tested strains. However, lectins modified the action of fluconazole, reducing the IC_{50} of the drug against *C. albicans*. Lectins were also able to discretely modulate the morphological transition of the tested strains.

Keywords Plant proteins · Opportunistic pathogens · Antifungal

Abbreviations

BVL	*Bauhinia variegate* Lectin
CA	*Candida albicans*
MC	Matrix concentration
CON A	*Canavalia ensiformis* Lectin
CON M	*Canavalia rosea* Lectin
Conbr	*Canavalia brasiliensis* Lectin
CT	*Candida tropicalis*
FCZ	Fluconazole
GC	Growth control
Helja	*Helianthus annuus* Lectin

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✉ Henrique Douglas Melo Coutinho
hdmcoutinho@urca.br

Victor Juno Alencar Fonseca
victorjunobiomedico@gmail.com

Ana Lays Braga
ana.lays@hotmail.com

Ray Silva de Almeida
rayalmeidasilva2306@gmail.com

Taís Gusmão da Silva
tais.gusmao@urca.br

Josefa Carolaine Pereira da Silva
carol.bio1881@outlook.com

Luciene Ferreira de Lima
luciene.ferreira@urca.br

Maria Helena Cruz dos Santos
santos.mariahelenacruz@gmail.cruz

Romério Rodrigues dos Santos Silva
romeriorodrigues95@hotmail.com

Claudener Souza Teixeira
claudener@gmail.com

Maria Flaviana Bezerra Morais-Braga
flavianamoraisb@yahoo.com.br

[1] Universidade Regional do Cariri, URCA, Cel Antônio Luis, Pimenta, Crato, CE 1161, 63105-000, Brazil

[2] Centro de Ciências Agrárias e da Biodiversidade, Universidade Federal Do Cariri, Crato, CE, Brazil

INCQS	Instituto Nacional de Controle de Qualidade em Saúde
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
PDA	Potato dextrose agar
PHA	*Phaseolus vulgaris* Lectin
ROS	Reactive oxygen species
SDA	Sabouraud dextrose agar
WSMoL	*Moringa oleifera* Lectin
YPD	Yeast extract peptone dextrose

Introduction

Over the years, the different factors linked to the diffusion and spread of invasive candidiasis have gradually changed worldwide, the main reason being the emergence of various *Candida* species (Ghazi et al. 2019), where there are already more than 200 yeasts of this genus (Brandt and Lockhart 2012), in which the pathogens *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and the most recent, *Candida auris* (Geddes-McAlister and Shapiro 2019) are included.

The incidence of these microorganisms varies between 2 and 14 per 100,000 people worldwide, and according to population surveys, at least 10–20% of those infected die. This problem has been observed both in developing and developed countries, reaching a high mortality rate globally (Lamoth et al. 2018). In Brazil, this number has been increasing every year. In 2017, 62 deaths from candidiasis were recorded, reaching 92 in 2019 adding all regions of the country (Brasil 2019).

It is remarkable that, although science and medicine have developed over the years, these microorganisms pose an increasing threat. Species such as *C. albicans* and *C. tropicalis* have characteristics that make them difficult pathogens to eradicate, such as the production of biofilms and their plasticity regarding morphology. Under favorable conditions, they can undergo a transition from their yeast form to hyphae, a phenomenon known as cellular morphogenesis, which is often associated with virulence, aiding in colonization, tissue invasion, and immune system evasion (Kadosh 2019; Kornitzer 2019; Sharma et al. 2019).

Moreover, some of their characteristics, such as the presence of efflux pumps and ability to form biofilm (Sharma et al. 2019) lead to increased fungal resistance to drugs, having the class of antifungal agents a limited number of drugs with different fungal components as targets (Maubon et al. 2014). The plasticity of fungal cells of the *Candida* genus has often been associated with their increased virulence, and for this reason, there is great interest in researching compounds capable of inhibiting these factors, being more favorable to reduce the virulence of the fungus, allowing the host immune system to eliminate the pathogen, an effective strategy in response to antifungal resistance (Kadosh 2019; Sharma et al. 2019).

Many of these responses can be found in nature, where living things must respond promptly to environmental stress, whether physical, chemical, or biological in nature, a way to confer protection against predators and/or pathogens. As part of these defense mechanisms are lectins, which can recognize and interact with carbohydrates present in the membranes of cells and microorganisms, promoting cell signaling that can confer greater resistance to pathogens in animals and plants (Lannoo and Van Damme 2014; Pinto et al. 2011). Legumes produce seeds rich in bioactive lectins, proteins that assist them in their defense, the main representative of these lectins being ConA, extracted from *Canavalia ensiformis* (Van Damme et al. 1998).

Lectins make reversible binding to specific free carbohydrates or cell wall polysaccharides and cell membrane glycoconjugates (De Hoff et al. 2009; Sharon 2007), and due to this binding to carbohydrates, also present in the cell wall or cell membranes of several microorganisms, they perform a variety of biological activities (Araújo-Filho et al. 2010).

Considering the promising antimicrobial effect of lectins, including already reported in the literature against viruses, (Gondim et al. 2019), bacteria (Procópio et al. 2017), protozoa (Castanheira et al. 2015), and fungi (Regente et al. 2014), this study aims to analyze the antifungal potential, intrinsic and combined to drug, of the lectins ConA and ConM, extracted from seeds of *Canavalia ensiformis* and *Canavalia rosea* (cited in the literature and lectin database by the synonymy *Canavalia maritima* Thouars) respectively, against standard strains of *C. albicans* and *C. tropicalis* strains, respectively, as well as their effect on inhibiting a virulence factor of the genus, the morphological transition.

Antifungal tests

Used strains and preparation of the materials

The fungal strains used were *C. albicans* (INCQS 40006—ATCC 10231) and *C. tropicalis* (INCQS 40042—ATCC 13803), obtained from the Culture Collection of the Instituto Nacional de Controle de Qualidade em Saúde (INCQS), Oswaldo Cruz Foundation. Aliquots were collected from them using a platinum loop and then solubilized in saline solution until a turbidity standard equivalent to 0.5 on the McFarland scale was obtained, except for the lectin effect test on the morphological transition of *Candida* spp, where the strains were inoculated in Yeast Extract Peptone Dextrose (YPD) medium—interlab, enriched with 5% serum and subsequently plated on Sabouraud Dextrose Agar (SDA)—KASVI.

The antifungal of choice was fluconazole—isofarma® at a concentration of 0.2%, equivalent to 2 mg/mL. For the tests, fluconazole was diluted in sterile distilled water to the matrix concentration of 1024 µg/mL. The lectins went through the same dilution process, taking care to homogenize the solution gently, without using a vortex. All culture media were weighed on precision scales and prepared according to the manufacturer, diluted in distilled water, except the Sabouraud Dextrose Broth—ISOFAR medium, which was prepared doubly concentrated.

Lectins purification

Seeds from *Canavalia ensiformis* and *Canavalia rosea* were ground to a fine powder in a coffee mill, and the soluble proteins were extracted at 298 K by continuous stirring with 0.15 M NaCl [1:10 (w:v)] for 1 h, followed by centrifugation at 10,000×*g* at 277 K for 20 min. Protein purification was carried out by the affinity chromatography protocol previously described by Teixeira et al. (2012), using a Sephadex G-50 column (10 × 50 cm) equilibrated with 150 mM NaCl containing 5 mM $CaCl_2$ and 5 mM $MnCl_2$. After washing of the unbound material in the equilibrium solution, the lectin was eluted from the gel with 100 mM glucose, pooled, exhaustively dialyzed against distilled water, and freeze-dried. Absorbance at 280 nm was used to estimate the protein concentration in all chromatographic fractions.

After the purification process, the lectins ware dialyzed with distilled water and lyophilized for the biological assays. The purity of the lectin sample was assessed by electrophoresis on 12.5% SDS-PAGE as suggested in Laemmli (1970).

Intrinsic activity of the antifungal effect of lectins and fluconazole

Flat bottom 96-well plates were used to perform the broth microdilution method as described by Javadpour et al. (1996). A serial dilution process was performed, and the concentrations of lectins and fluconazole ranged from 512 to 0.5 µg/mL. The last wells of the plates were reserved for growth control of the microorganisms. Dilution controls of the products (using saline solution instead of inoculum) and sterility of the medium were also conducted. The plates were incubated at 37 °C for 24 h. After this period, the growth of microorganisms was observed, as evidenced by the presence of turbid medium on the plates. For quantitative tests, the plates were taken for reading in an ELISA spectrophotometer apparatus (Termoplate®), with a wavelength of 630 nm (Morais-Braga et al. 2016). The results provided the minimum inhibitory concentration (MIC) of the tested products, as well as the IC_{50}. The tests were performed in quadruplicate.

Determination of minimum fungicidal concentration

To determine whether the products alone and in combination were able to affect the viability of *Candida* cells, the minimum fungicidal concentration (MFC) was determined. Using a pipette, 5 µL from each well of the MIC test plate (except for the sterility control and product dilution) were transferred to Petri dishes containing SDA medium, distributing according to the guide card at the bottom of the plate. After 24 h of incubation, the plates were inspected for any *Candida* colony formation (Ernst et al. 1999). The concentration at which there was no growth of fungal colonies will be considered the MFC of the product.

Combined activity of lectins with fluconazole

To execute the test, the lectins were used in subinhibitory concentration (MC/8, where MC is the Matrix Concentration of the evaluated products) according to the methodology used by Coutinho et al. (2008), with minor modifications. The commercial drug (fluconazole) was used in the drug combination test in serial dilution at concentrations ranging from 512 to 0.5 µg/mL. The last wells of the plates were reserved for growth control of the microorganisms. Dilution controls of the product in combination with fluconazole were also performed, in addition to the sterility control of the medium. The whole test was performed in quadruplicate and the plates were incubated at 37 °C for 24 h. The reading was performed in an ELISA spectrophotometry apparatus (Termoplate®) with a wavelength of 630 nm and the results will be used to obtain a cell viability curve (Morais-Braga et al. 2016).

Effect of lectins on the morphological transition of *Candida* spp.

Sterile micromorphological chamber slides were prepared for yeast observation. Three milliliters of Potato Dextrose Agar (PDA) medium—Becton Dickinson & Co. USA, depleted by dilution were added to the chambers, containing the natural product concentrations (lectins) MC and MC/4, a concentration, which according to Houghton et al. (2007), it is clinically relevant (256 µg/mL) and another which would be the matrix concentration (1024 µg/mL). Aliquots of the inocula were taken from the Petri dishes to make two parallel streaks on the solid medium, which were then covered with a sterile coverslip. The chambers were incubated for 24 h (37 °C) and inspected under a light microscope with a 10 × objective. A camera was attached to the L-2000I-TRINO/6633—Bioval microscope to capture images. A yeast growth control (hyphae stimulated by depletion) was performed, as well as a control with the antifungal

drug fluconazole for comparison purposes. The assays were produced according to Sidrim and Rocha (2010) and Mendes (2011), with some modifications.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 software (Free trial GraphPad Software, Inc., La Jolla, CA, USA). Data were expressed as geometric means. Statistical significance was assessed with a two-way ANOVA test followed by Bonferroni post hoc test (where $p < 0.05$ and $p < 0.0001$ are considered significant and $p > 0.05$ not significant).

To evaluate virulence, the total area of the streaks was measured using ToupView software (ToupTek Photonics®—China), checking the areas where there was hyphae growth. Next, measurements were taken of all the hyphae filaments found in five random areas in each streak, for each concentration. The average length of the hyphae filaments was taken and analyzed by ANOVA followed by Bonferroni correction for multiple comparisons, comparing the values according to the concentration of the product (Carneiro et al. 2019).

Results

Lectins purification

ConA and ConM ware purified, as described by Teixeira et al. (2012). The chromatograms showed two peaks, the first (PI) corresponding to unbound protein fraction and the second (PII) corresponding to the retained protein fraction (Fig. 1A and C). In SDS-PAGE, ConA showed three bands (Fig. 1B), the first band corresponding to α-chain (25.5 kDa), the second to β-chain (14 kDa), and the third to γ-chain (12 kDa). ConM two bands (Fig. 1D) the first band corresponding to α-chain (25.5 kDa), the second to γ-chain (12 kDa).

Antifungal tests

After the 24-h period in the oven, the 96-well plates were removed and analyzed for turbidity of the medium. The results revealed fungal growth in all wells. After analysis in ELISA on the 630 nm filter to determine the Minimum Inhibitory Concentration (MIC), inhibition of fungal growth could not be observed in any of the concentrations tested (Table 1), both for lectins and fluconazole, although the latter achieved slightly better values, but without significance, indicating that, at these concentrations, lectins do not present antifungal activity when used

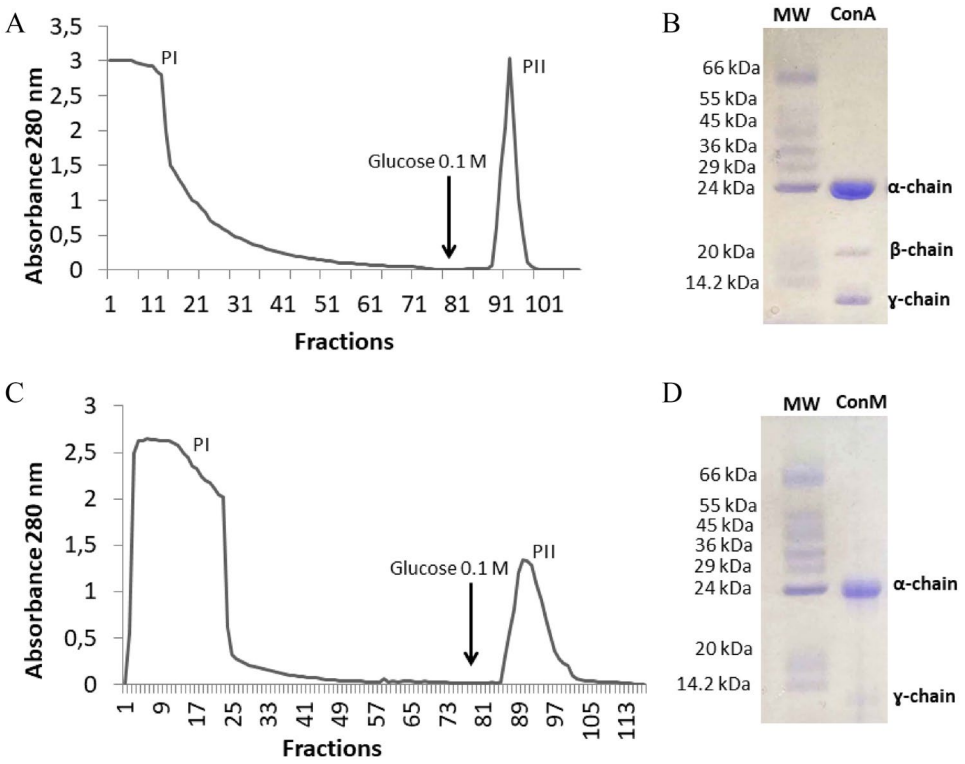

Fig. 1 ConA and ConM purification by affinity chromatography. **A** Chromatogram of Canavalia ensiformis crude extract in Sephadex G-50 column. **B** SDS-PAGE from the ConA. Lane 1: Molecular weight marker (MW); Lane 2: PII (Purified ConA). **C** Chromatogram of Canavalia rosea crude extract in Sephadex G-50 column. **D** SDS-PAGE from the ConM. Lane 1: Molecular weight marker (MW); Lane 2: PII (Purified ConM). Molecular weight marker: bovine serum albumin 66 kDa; glutamic dehydrogenase, 55 kDa; ovalbumin, 45 kDa; glyceraldehyde-3-phosphate dehydrogenase, 36 kDa; carbonic anhydrase, 29 kDa; trypsinogen, 24 kDa; trypsin inhibitor, 20 kDa; α-lactalbumin, 14.2 kDa

Table 1 Inhibitory concentration of 50% of microorganisms (µg/mL) by lectins and fluconazole alone and combined

Microorganisms	IC_{50} of microorganisms for evaluated compounds				
	CON A	CON M	FCZ	CON A + FCZ	CON M + FCZ
CA INCQS 40006	539,1	607,2	432,2	19,6	17,6
CT INCQS 40042	502,5	405,5	275,1	567,9	575,9

FCZ Fluconazole, *CA Candida albicans*, *CT Candida tropicalis*, *CON A* lectin obtained from *Canavalia ensiformis*, *CON M* lectin obtained from *Canavalia rosea*, *INCQS* Instituto Nacional de Controle de Qualidade em Saúde

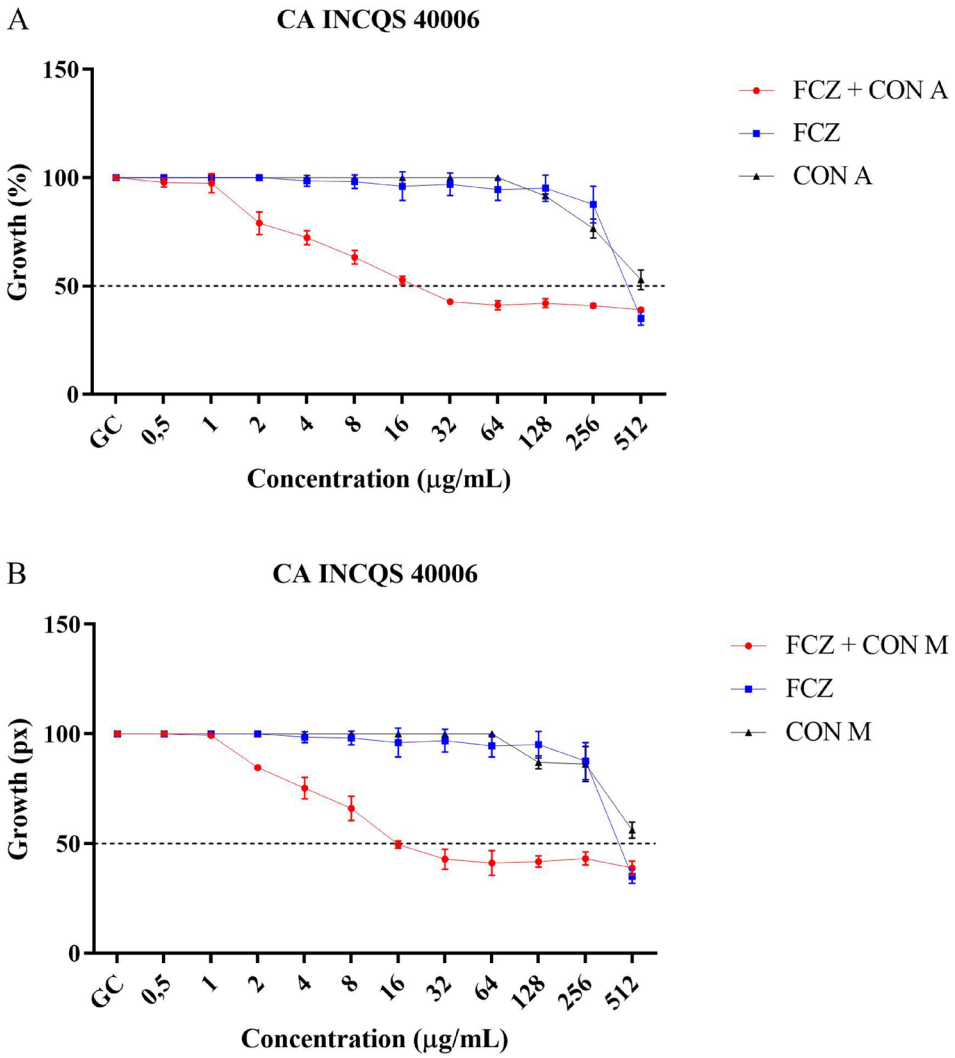

Fig. 2 Modulation of the antifungal effect of fluconazole complexed with Con A (**A**) and Con M (**B**) against *C. albicans* 40006. CA INCQS 40006: *Candida albicans* ATCC 10231; *Con A Canavalia ensiformis* lectin; *Con M Canavalia rosea* lectin, *FCZ* Fluconazole, *FCZ* + *Con A* Fluconazole + *Canavalia ensiformis* lectin, *FCZ* + *Con M* Fluconazole + *Canavalia rosea* lectin; *GC* Growth control, *INCQS* Instituto Nacional de Controle de Qualidade em Saúde

by themselves. Due to the absence of significant inhibition of lectins throughout the concentrations evaluated, the MFC was performed only with the antifungal fluconazole, revealing a fungistatic action at the concentration of 512 µg/mL and no fungicidal effect (data not shown).

Canavalia lectins modify the action of fluconazole

The association of lectins at subinhibitory concentrations with fluconazole increased the activity of the antifungal against *C. albicans* 40006, reducing by more than 50% the

amount of antifungal required to inhibit 50% of the fungal growth of *C. albicans* at concentrations ranging from 16 to 512 µg/mL, while for *C. tropicalis* the inhibition was slight at first and then stagnated, indicating that the yeasts adapted to the combined treatment (Figs. 2 and 3).

Action of lectins on the morphological transition of *Candida* spp.

The morphological transition from yeast to filamentous in the growth controls with *C. albicans* and *C. tropicalis* could be observed at all edges of the streaks (100% growth). Lectin solutions were used at MC and MC/4 concentrations (1024 µg/mL and 256 µg/mL, respectively), and growth was observed at all edges of the streaks, except at the MC/4 concentration of ConM against *C. albicans* (55.6% growth). In the controls with fluconazole used in the same concentrations, a 100% inhibition in the morphological transition of the fungi was observed, with a discrete growth only in MC/4 of *C. albicans*, which showed 100% growth in all edges of the streak, but with a reduction in the length of the hyphae of

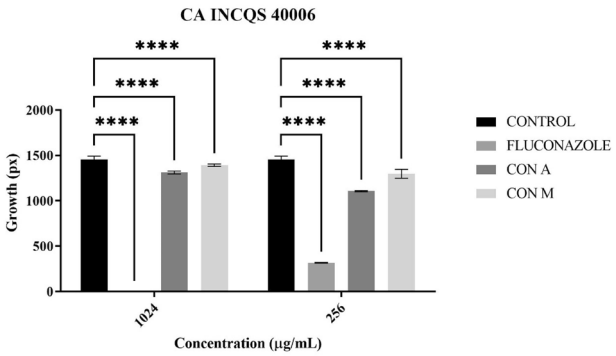

Fig. 4 Graphs of the effect on the morphological transition of ConA and ConM lectins against *Candida albicans* compared to growth control. CA INCQS 40006: *Candida albicans* ATCC 10231; *Con A Canavalia ensiformis* lectin, *Con M Canavalia rosea* lectin, *INCQS* Instituto Nacional de Controle de Qualidade em Saúde

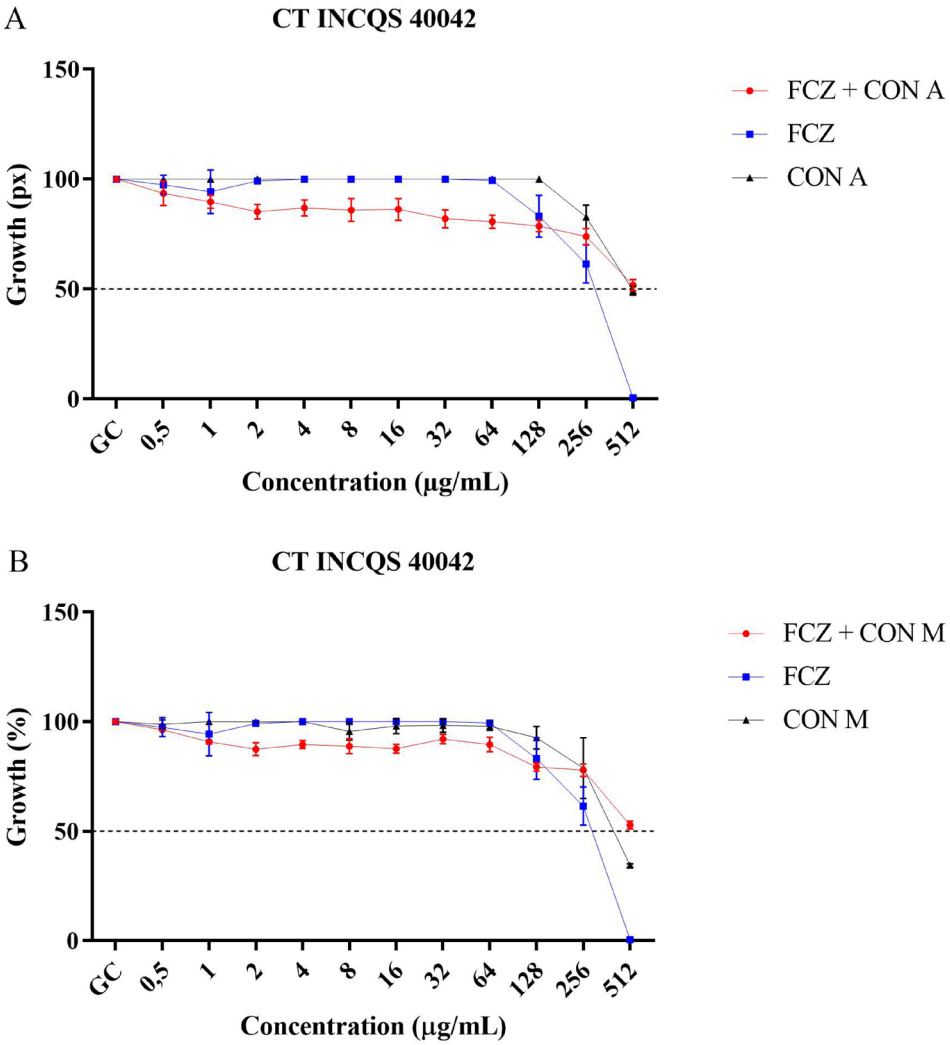

Fig. 3 Modulation of the antifungal effect of fluconazole complexed with Con A (**A**) and Con M (**B**) against *C. tropicalis* 40042. CT INCQS 40042: Candida tropicalis ATCC 13803; *Con A* Canavalia ensiformis lectin, *Con M* Canavalia rosea lectin; *FCZ* Fluconazole, *FCZ* + *Con A* Fluconazole + Canavalia ensiformis lectin, *FCZ* + *Con M* Fluconazole + Canavalia rosea lectin, *GC* growth control, *INCQS* Instituto Nacional de Controle de Qualidade em Saúde

more 78% compared to the growth control (Supplementary material S1 and S2).

The results expressed in Fig. 4 show that, compared to the growth control, the products were statistically relevant, without completely inhibiting the transition, but reducing the final length of the hyphae. However, it was observed that at lower concentrations of MC/4, the results were more promising than at higher concentrations of the product (MC), except for the MC of ConM against *C. tropicalis*, which showed a considerable reduction of 42.6% in hyphae length, a value almost three times greater than that presented in MC/4 (14.7%) (Figs. 4 and 5).

Discussion

The use of natural products has provided alternatives to treatments pre-established by medicine. It is possible to find in nature several compounds capable of acting at the same level as commercial drugs, and with fewer adverse effects. Coumarins are secondary metabolites found in several plants and which have a remarkable anticancer effect reported in the literature. Considering that chemotherapy and radiation are the main treatments in these cases, coumarins may represent a less harmful alternative to the patient (Ahmed et al. 2020).

Although these substances have reports of hepatotoxicity, Akkol et al. (2020) states that their analogs, obtained by molecular manipulations, are relatively safe. Other secondary metabolites, such as flavonoids, can be used to palliate the adverse effects caused by chemotherapy treatment, such as diarrhea, mucositis, neuropathic pain and others (Fernandez et al. 2021).

In a work by Goni et al. (2021), the plant *Aglaonema hookerianum* is used to treat various diseases, including sexual disorders and depression. Their data show that the extract of this plant was able to treat anxiety behavior in mice, at concentrations of 200 and 400 mg/kg. Animals treated at different doses showed improvement in sexual arousal in male mice.

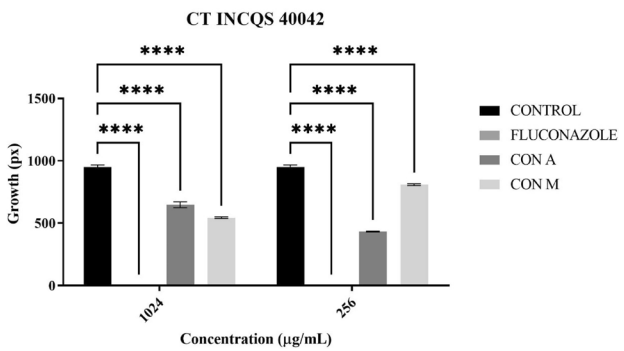

Fig. 5 Graphs of the effect on the morphological transition of ConA and ConM lectins against *Candida tropicalis* compared to growth control. CT 40042: *Candida tropicalis* ATCC 13803; *Con A Canavalia ensiformis* lectin, *Con M Canavalia rosea* lectin, *INCQS* Instituto Nacional de Controle de Qualidade em Saúde

At the current moment, when the world faces the Covid-19 pandemic, many researches have been carried out with substances of natural origin. The main reason is, in addition to the emergency treatment of this disease, an alternative to the cytotoxicity presented by antivirals (Tallei et al. 2021). Most of the adverse effects presented by these drugs are related to gastrointestinal disorders, such as hydroxychloroquine, favipiravir, remdesivir and others (Ağagündüz et al. 2021). Polyphenols, such as those obtained from green tea, are reported to inhibit SARS-CoV-2 infectivity. In addition, its extracts have immunomodulatory activity, by influencing the proliferation of T lymphocytes and the production of cytokines (Tallei et al. 2021).

In research carried out by Fonseca et al. (2022), lectins of natural origin are pointed out for having remarkable antimicrobial activity. Due to their ability to selectively bind to carbohydrates, they can interact in various ways on the cell wall of bacteria, fungi and protozoa. Lectins with antifungal activity are mostly extracted from plants. These proteins are believed to be involved in the defense system of plants, which in their natural habitat, they need to defend themselves against insects, bacteria, and fungi (Yan et al. 2005). As a way of propagation and survival of the species, these proteins are stored mainly in reproductive organs, fruiting bodies, and tubers, but they are not restricted to this, and may also be present in several other plant tissues (Hasan et al. 2014; Hiremath et al. 2020).

In research conducted by Klafke et al. (2013), lectins extracted from seeds of *Canavalia brasiliensis* (Conbr), *Mucuna pruriens*, *Clitoria fairchildiana*, *Dioclea virgata*, and *Bauhinia variegata* (BVL) were tested against isolates of *C. albicans*, *C. tropicalis*, *Candida parapsilosis*, *Cryptococcus gattii*, *Cryptococcus neoformans*, *Malassezia pachydermatis*, *Rhodotorula* sp. and *Trichosporon* sp, with significant activity only against *C. parapsilosis* at concentrations ranging from 0.97 to 125 µg/mL. In our research, the lectins extracted from *Canavalia ensiformis* (ConA) and *Canavalia rosea* (ConM) seeds were tested against standard strains of *C. albicans* and *C. tropicalis*, is not showing antifungal activity at the concentrations tested.

It is important to note that both ConA and ConM lectins have an affinity for mannose and glucose (Araújo-Filho et al. 2010), components of the cell wall, but lack affinity for N-acetylglucosamine, which constitutes the chitin, a homopolysaccharide found in insect exoskeletons, fungal cell walls, nematode eggs, marine diatoms, and the shells of crustaceans and zooplankton (Chen et al. 2018). Lectins that bind to chitin seem to have more promising fungicidal activity. This is the case of *Phaseolus vulgaris* lectin (PHA),

which inhibited the growth of *Coprinus comatus*, *Fusarium oxysporum*, and *Rhizoctonia solani* (Ye et al. 2001). According to Ye et al. (2001), the red bean lectin showed some degree of structural similarity to chitinases. This may account in part for its antifungal activity because chitinases are known to adversely affect hyphae growth, leading to cell wall disruption, the release of chitin oligosaccharides from the cell wall, and cytoplasm leakage.

Another chitin-specific lectin is extracted from *Moringa oleifera* seed (WSMoL), which showed fungicidal activity at concentrations of 80, 20, 40 and 80 µg/mL for *C. albicans*, *C. glabrata*, *Candida krusei* and *C. parapsilosis*, respectively (L. M. M. Santos et al. 2020a, b). The absence of antifungal activity of ConA and ConM, compared to the activities reported in the literature for other lectins, may be due to the absence of the interaction of ConA and ConM with chitin, and the variation in cell wall carbohydrate composition among fungal species (Klafke et al. 2013).

Modifying activity was observed when subinhibitory concentrations of ConA and ConM lectins associated with fluconazole were used, reducing the IC_{50} of the antifungal by more than 50% against *C. albicans*. Combination drug therapy is commonly recommended in the treatment of infectious agents in intensive care units, considering that not all pathogens are sensitive to monotherapy (Joung et al. 2016). In agreement with the data were obtained, the research conducted by Ferreira et al. (2018) also showed antifungal activity in the combination of ApuL lectin, extracted from *Alpinia purpurata*, with fluconazole, reducing the IC_{50} by eightfold. Santos et al. (2020a, b) proposes that lectins act by delivering the drug to target cells through carbohydrate recognition in the membrane, which leads to drug release, facilitating the entry of the antifungal into the microbial cytoplasm. The lack of activity in combination therapy against *C. tropicalis* may be due to the high resistance to azolic compounds that this species has, specifically to fluconazole (Zuza-Alves et al. 2017). In addition, due to the hydrophobic nature of the carbohydrate recognition domain of lectin, it is possible that the interaction between *Canavalia* lectins and fluconazole occurs at another site, due to the hydrophilic nature of fluconazole (Kennedy et al. 1995).

It is interesting to note that although fluconazole by itself did not show positive results at the concentrations tested in MIC, it was able to completely inhibit the dimorphism presented by the species tested at the higher concentrations of the product. This occurs because ergosterol biosynthesis is the target of the class of azole antifungals, which suggests a relationship between components of the ergosterol biosynthesis pathway with fungal filamentation (Kadosh 2019).

Studies have reported that many small molecules are able to modulate morphogenesis in *C. albicans* and that these acts directly or indirectly on the pathways of morphological transition from the yeast to filamentous phase (Shareck and Belhumeur 2011; Sharma et al. 2019). The most reported example in the literature is farnesol, a quorum sensing molecule that is produced by fungi and can block filamentous growth by acting on the cAMP-PKA pathway and other regulatory factors responsible for hyphae formation (Shareck and Belhumeur 2011; Sharma et al. 2019). It was shown that *C. albicans* cells treated with azoles produced high levels of farnesol, a direct consequence of the sterol biosynthetic intermediate accumulation that indirectly stimulates the overproduction of farnesol (Hornby and Nickerson 2004).

Although the mechanisms by which lectins act on fungal cell walls is not completely elucidated, there are reports of these proteins having activity in morphological transition and antibiofilm, a factor directly linked to dimorphism in species such as *C. albicans*. This is the case of lectin extracted from *Helianthus annuus* (Helja) seeds, which inhibited the morphological change from yeast to filamentous in *C. albicans*, an important attribute for the pathogenicity of this microorganism. Helja also inhibited the development of biofilm formed by *C. albicans* by 40% when used in the early phases (adhesion to surface) of biofilm formation and by 30% in the intermediate phase (attached and secreting extracellular matrix components) (Del Rio et al. 2019).

In the present study, it is possible to see the confirmation of what has been observed in the literature that lectins can act on the morphological transition of *C. albicans* and *C. tropicalis*, having presented statistically significant results for MC and MC/4 concentrations of ConA and ConM. However, the results were better when observed at MC/4 and this may be related to the production of reactive oxygen species (ROS). Although these compounds can be toxic to many microorganisms, recent studies have shown that at subtoxic levels, H_2O_2 promoted hyphae development and that *C. albicans* may itself generate these ROS, contributing to its morphogenesis, a condition in response to a stress stimulus (Rossi et al. 2017).

It is also possible that this result is related to the affinity of these lectins for polysaccharides that make up the cell wall of *Candida* spp. such as mannose, glucose, and chitin (Hameed et al. 2018). This type of affinity may act competitively to other ligands of these polysaccharides. Tunicamycin, for example, has a blocking effect on the formation of N-acetylglucosamine, significantly altering the glycosylation process of proteins (Omeara et al. 2015). According to Kadosh (2019), these findings suggest that polysaccharide remodeling on the cell surface of *C. albicans* may play a crucial role in its morphogenesis.

Considering that resistance to antifungal drugs has become an increasingly present reality, our results show that ConA and ConM lectins can be used in combined therapy with azole compounds to treat patients affected by *Candida* spp.

Conclusion

Although ConA and ConM did not show antifungal activity by themselves, they showed an important modify action with the antifungal drug fluconazole, mainly against *C. albicans*. They were also able to reduce the development of hyphae during the morphological transition of *C. albicans* and *C. tropicalis*. It is necessary to elucidate the mechanisms by which lectins can act and to complement the results obtained with antibiofilm tests since this virulence factor is directly linked to the fungal dimorphism of these species. It is interesting to note that the best results for the morphological transition test were obtained from the lowest concentrations. This may represent an adaptation of the fungus by the level of stress exerted by the high concentration of the substances, which may have led to the expression of genes that act directly or indirectly to promote the filamentous form. More specific tests to elucidate these mechanisms should be carried out and, given the growing interest of medicine in obtaining new substances with different pharmacological targets, these lectins may represent great importance for the development of new drugs.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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