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Ethanolic extract of *Geastrum saccatum* (Basidiomycota: Geastraceae) has inhibitory power in the development of strains of *Candida* spp.?

EXTRATO ETANÓLICO DE GEASTRUM SACCATUM (BASIDIOMYCOTA: GEASTRACEAE) POSSUI PODER INIBITÓRIO NO DESENVOLVIMENTO DE CEPAS DE CANDIDA SPP.?

EL EXTRACTO ETANÓLICO DE GEASTRUM SACCATUM (BASIDIOMYCOTA: GEASTRACEAE) TIENE UN PODER INHIBIDOR EN EL DESARROLLO DE CEPAS DE CANDIDA SPP.?

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Abstract

Due to the appearance of isolated resistant cases of *Candida* spp. to known antifungal drugs, there is a demand and concern in finding and testing new substances for the treatment of candidiasis. This study aimed to evaluate the inhibitory potential of the fungus *Geastrum saccatum* (Basidiomycota: Geastraceae) over some *Candida* (Ascomycota: Debaryomycetaceae) species. Herein, we tested ethanolic extracts of basidiomes of the species *G, saccatum*, collected in an area of Environmental Preservation belonging to the UniEVANGÉLICA University Center of Anápolis, located in the city of Anápolis, against *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis*. The analysis on inhibitory activity was performed by means of macrodilution test and plaque sensibility test in cells of *C. parapsilosis*, *C. albicans* and *C. tropicalis*, subjected to concentrations of 2%, 1% and 0,5% of ethanolic extract of *G. saccatum*. According to obtained results, in different concentrations of ethanolic extract of *G. saccatum* added to plates containing *C. albicans*, *C. parapsilosis* and *C. tropicalis*, it was not possible to observe any inhibition of the growth of the fungus. In macrodilution tests there was also no inhibition of the growth of *C. albicans*, *C. parapsilosis* and *C. tropicalis* by the fungus *G. saccatum* in any of the tested concentrations, making it impossible to determine the minimum inhibitory concentration. Both tests were confirmatory for the negativity of antifungal potential of *G. saccatum* on *Candida* species.

Keywords: Antifungals. *Candida albicans*. *Candida parapsilosis*. *Candida tropicalis*. Gasteroid fungus.

Resumo

Devido ao aparecimento de isolados resistentes de *Candida* spp. a fármacos antifúngicos conhecidos, há uma demanda e preocupação em se encontrar e testar novas substâncias para o tratamento de candidíase. Este trabalho teve como objetivo avaliar o potencial inibitório do fungo *Geastrum saccatum* (Basidiomycota: Geastraceae), sobre espécies de *Candida* (Ascomycota: Debaryomycetaceae). Neste estudo, foi realizado teste de extratos etanólicos de basidiomas da espécie *Geastrum saccatum* coletados em uma área de Preservação Ambiental pertencente ao Centro Universitário Anápolis UniEVANGÉLICA, localizada na cidade de Anápolis, contra *Candida albicans*, *Candida parapsilosis* e *Candida tropicalis*. A avaliação de atividade inibitória foi realizada por meio de teste de macrodiluição e teste de sensibilidade em placas em células de *C. parapsilosis*, *C. albicans* e *C. tropicalis* submetidas a concentrações de 2%, 1% e 0,5% do extrato etanólico de *G. saccatum*. De acordo com os resultados obtidos, em diferentes concentrações de extrato de *G. saccatum* acrescidas em placa contendo *C. albicans*, *C. parapsilosis* e *C. tropicalis*, não foi possível observar inibição do crescimento do fungo. Em testes de macrodiluição também não transcorreu inibição do crescimento de *C. albicans*, *C. parapsilosis* e *C. tropicalis* pelo fungo *G. saccatum* em nenhuma das concentrações testadas, impossibilitando determinar a concentração inibitória mínima. Ambos os testes foram comprobatórios para a negatividade do potencial antifúngico de *G. saccatum* sobre as espécies de *Candida*.

Palavras-chave: Antifúngicos. *Candida albicans*. *Candida parapsilosis*. *Candida tropicalis*. Fungo gasteroide.

Resumen

Debido a la aparición de *Candida* spp. resistente medicamentos antifúngicos conocidos, existe una demanda y preocupación por encontrar y probar nuevas sustancias para el tratamiento de la candidiasis. Este trabajo tuvo como objetivo evaluar el potencial inhibitorio del hongo *Geastrum saccatum* (Basidiomycota: Geastraceae) sobre especies de *Candida* (Ascomycota: Debaryomycetaceae). En este estudio, se realizó un ensayo con extractos etanólicos de basidiomas de la especie *Geastrum saccatum* colectados en un área de Preservación Ambiental perteneciente al Centro Universitário Anápolis UniEVANGÉLICA, ubicado en la ciudad de Anápolis, contra *Candida albicans*, *Candida parapsilosis* y *Candida tropicalis*. La evaluación de la actividad inhibitoria se realizó mediante prueba de macrodilución y prueba de sensibilidad en placas en células de *C. parapsilosis*, *C. albicans* y *C. tropicalis* sometidas a concentraciones de 2%, 1% y 0,5% del extracto etanólico de *G. saccatum*. De acuerdo con los resultados obtenidos, a diferentes concentraciones de extracto de *G. saccatum* adicionado a placas que contenían *C. albicans*, *C. parapsilosis* y *C. tropicalis*, no fue posible observar inhibición del crecimiento del hongo. En los ensayos de macrodilución tampoco se produjo inhibición del crecimiento de *C. albicans*, *C. parapsilosis* y *C. tropicalis* por parte del hongo *G. saccatum* en ninguna de las concentraciones ensayadas, imposibilitando determinar la concentración mínima inhibitoria. Ambas pruebas confirmaron la negatividad del potencial antifúngico de *G. saccatum* sobre especies de *Candida*.

Palabras clave: Antimicóticos. *Candida albicans*. *Candida parapsilosis*. *Candida tropicalis*. Hongo gasteroide.

Introduction

The species diversity in the Fungi Kingdom is estimated at 2.2 to 3.8 million species (HAWKSWORTH; LÜCKING, 2017), which inhabit diverse ecological niches and, just as plants and animals, have various ecosystem roles of extreme importance

for the balance of biological systems. Thus, they have great importance in the ecological and also in economic perspectives (MORAES; PAES; HOLANDA, 2009). Due to the cellular, genomic, and metabolic complexity of fungal life stories, the evolution favored a great morphological and functional diversity among fungi, encompassing convergent and divergent evolutive processes (NAGY; KOVÁCS; KRIZSÁN, 2018, NARANJO-ORTIZ; GABALDÓN, 2020). Some species are used for pharmacological purposes, such as anti-atherosclerotics, anti-bacterials, anti-diabetics, anti-parasitics, anti-tumors, antivirals, anti-inflammatories, anti-hypertensives, hepatoprotectives and immune system modulators (ABREU; ROVIDA; PAMPHILE, 2015). Some species areconsidered pathogenic and are capable of causes diseases in the human beings. Other species are opportunistic pathogens and can cause disease only when there is a change in the host's immune system, such as the genus *Candida* Berkhout (Ascomycota: Debaryomycetaceae), which has increased its incidence significantly over the years and has a poor prognosis with high mortality rates in bedridden and immunocompromised patients (BROWN, 2011).

The genus *Candida* Berkhout

The genus *Candida* (Ascomycota: Debaryomycetaceae) was proposed by Berkhout (1923), and currently is represented by about 200 species that can be found in the normal microbiota of the human body, plus other environments, manifesting in several human body niches, such as: mouth cavity, folds in the skin, vagina, penis, oropharynx, bronchial secretions, feces and urine (ÁLVARES; SVIDZINSKI; CONSOLARO, 2007). Species of *Candida* have a dimorphic life cycle, with their yeast phase spent inhabiting commensally the body niches aforementioned (HARIDY *et al.*, 2018).

Candida is composed by yeasts, belonging to eukaryotic microorganisms and are free of photosynthesizing pigmentation, as their cells are composed of phospholipid plasmatic membrane and chitin. Its nutrition is based on the absorption of carbon sources from the environment, due to its cell's walls being rigid, unable to perform phagocytosis (AGUIAR, 2007).

There are approximately 15 species of *Candida* that are pathogenic to humans, but more than 90% of cases of candidiasis, the infection caused by *Candida* proliferation, reported are associated with the five most common species: *C. albicans* (C.P. Robin) Berkhout, *C. glabrata* (H.W. Anderson) S.A. Mey. & Yarrow, *C. krusei* (Castell.) Berkhout, *C. parapsilosis* (Ashford) Langeron & Talice and *C. tropicalis* (Castell.) Berkhout. The majority lies in cases of *C. albicans*, with approximately 46.3% of cases of fungal diseases (DADAR *et al.*, 2018).

Due to the increase in immunosuppressed people, several factors that lead to a weakening of the immune system correlate with high numbers of human *Candida* infections (BORST, 2002). The infection can be detected in completely healthy people

in whom it is less severe in comparison to debilitated patients. Regarding healthy people, it is related to cases of infection in the mucosas and skin. However, in debilitated patients, the infection can be characterized by systematics, in which there are chances of a spread of the infection that generates complications in the body (COLOMBO; GUIMARÃES, 2003).

There is an increasing fungal resistance of the genus *Candida* to antifungal drugs used for the treatment of the disease. Species resistant to the amphotericin B have been reported, such as *C. lusitaniae* Uden & Carmo Souza, *C. guilliermondii* (Castell.) Langeron & Guerra, *C. inconspicua* (Lodder & Kreger) S.A. Mey. & Yarrow, *C. kefyr* (Beij.) Uden & H.R. Buckley ex S.A. Mey. & Ahearn, and *C. rugosa* (H.W. Anderson) Diddens & Lodder. Other antifungals as the azole, to which *C. glabrata*, *Candida dubliniensis* D.J. Sullivan, Western., K.A. Haynes, Dés.E. Benn. & D.C. Coleman, and *C. krusei* exhibit resistance, and species such as *C. guilliermondii*, and *C. parapsilosis*, in which equinocandins act, are also showing resistance (PAULA; RUIZ, 2017). *C. parapsilosis* and *C. tropicalis* present resistance mechanisms to azole and echinocandins, the latter being due to mutations in the FKS genes, resulting in a decrease in sensitivity to the antifungal (VIEIRA; NACIMENTO, 2017).

Candida albicans **Berkhout**

This species is considered as good strategist by its mechanism of relating to immune system cells. Thus, *C. albicans* induces macrophage pyroptosis, avoiding the attack of immune cells, and this occurs due to its morphogenetic exchange coupled to cell wall reform after phagocytosis by macrophages. There are at least 98 genes that do not participate in macrophage filamentation, but are necessary for the cell death of macrophages in the immune system affected by *C. albicans* (O'MEARA *et al.*, 2018).

The fundamental reproduction strategyof the species is clonal, but there are indications that sexual reproduction also occurs, found in the natural environment, but not found in laboratorial circumstances. Under natural growth conditions, the strains can reproduce both sexually and in low parasexual nature (TAVANTI *et al*., 2004). The species has a system of modifying its yeast phase to hyphae phase without going through the pseudo-hyphae phase (GOW, 1997). In pathogenicity, the growth of hyphae is what allows penetration into the tissues of the host (SZABO; MACCALLUM, 2011).

This species is highly tolerable to pH variation and develops in environments with pH differences between 2 to 10 due to its ability to change extracellular pH, changing its morphology. With alkaline and/or acidic changes happening to a neutral environment, modifications occur to the fungus in its process from the yeast phase to the hyphae phase, and consequently the growth of the hyphae. This mechanism can

be considered as an evolutionary adaptation that allows a greater virulence for the species, as well as permanence and maintenance in the host (VYLKOVA *et al.*, 2011).

Candida tropicalis Berkhout

This species is formed by pseudomycelium cells or ellipsoidal shoots, organized by long and branched elements with isolated conidia, in agglomerated or short chains (CHAI; DENNING; WARN, 2010). It is characterized only by pseudo-hyphae form, but in rare conditions, it is capable of form true hyphae (SUZUKI; MIYAMAE; ISHIDA, 1991; BUTLER *et al.*, 2009). *Candida tropicalis* is designated as a polymorphic species, with various morphological forms, comprising yeast, pseudo-hyphae, and true hyphae (LOPES; VAINSTEIN; SCHRANK, 2018).

The species deals better with tropical climates, having a higher prevalence in countries with favorable characteristics for the species proliferation (CHAI; DENNING; WARN, 2010). In addition to humans, *C. tropicalis* is found in the environment (e.g., water and sand beaches) and in other animals (ZUZA-ALVES *et al*., 2019).

Reproduction of the species has been reported as asexual, but under favorable conditions, reproduction can occur sexually. Even at 37°C, *C. tropicalis* showed efficiency in the exchange of genetic material, in which there is a probability that the strains undergo sexual exchanges during infection and colonization of the host. Due to this discovery, comes the possibility of correlating sexual reproduction to an indication that the species becomes more virulent (PORMAN *et al*., 2011).

Candida parapsilosis Langeron & Talice

This species is mainly detected in human skin, and can be found in patients with infections, skin lesions and vaginitis (GÁCSER *et al.,* 2007). Aside from humans, the species can be found in the environment and other animals (WEEMS, 1992). The morphology in its saprophytic phase is characterized by oval yeast cells (sometimes round or cylindrical), and in the parasitic phase is characterized by yeast or pseudo-hyphae, not having true hyphae (KIM; BISSATI; MAMOUN, 2006; MARRETTO, 2014). This species has an exclusively sexual reproduction (BARBEDO; SGARBI, 2010).

Candidiasis caused by *C. parapsilosis* can be associated by factors of hyperbolic use of venous catheters in newborns, parenteral nutrition and patients who have undergone transplantation processes, which can be explained by the compatibility that *C. parapsilosis* has to parenteral nutrition and intravenous devices, also due to the ability to proliferate in high richness of glucose and lipids present in the bloodstream (ALMIRANTE, 2006; HAJJEH; WARNOCK, 2003).

The genus *Geastrum* Pers.

The genus *Geastrum* was proposed by Persoon (1801) and is currently a member of the family Geastraceae in the Fungi Kingdom. This is one of the most diverse gasteroid genus in Geastraceae and, with the exception of Antarctica, it has distribution in all continents. According to the fungal collection of the Royal Botanic Gardens Kew, approximately 60 species have been catalogued, in which the species *Geastrum saccatum* Fr. found in Uruguay is the oldest species in the collection, dated 1832. However, recent data present an estimate of described species about 100 to 120 worldwide species that are found naturally in the environment (ZAMORA *et al*., 2014). In Brazil, there are 73 species of the genus, and the species *G. saccatum*, one of the most common of the genus, is distributed at least in six Brazilian states (TRIERVEILER-PEREIRA; BASEIA, 2009; FREITAS-NETO *et al*., 2023).

The morphology of this genus is characterized by petaloid dehiscence, having basidiomes, in which before its maturation the specimen is in globular shapes and during maturation the outer layers separate from the inner axis and distance from its basidiomes, forming its typical sporomes, which finally resemble a star (because of this, the fungi of this genus are known as earthstars). The fungus has a stellate basidiome; the peridium (membrane lining the gleba), that can present up to three layers: exoperidium (outermost layer), mesoperidium (middle layer) and endoperidium (innermost layer); powdery gleba (contains basidiums and basidiospores); subiculum (structure that penetrates underground) with the appearance of crust, wool or net, which grows before the basidiome and the formation of ostiolus at the apex of the sporome (LIMA, 2018). Inside the mature endoperidium there is a well-developed capillary filling composed of a powdery mass of simple and unbranched spores, with filaments trapped in its inner wall, a structure that assists in the dispersion of spores and the disposal of residual materials (CUNNINGHAM, 1944).

Geastrum saccatum Fr.

This species is considered cosmopolitan, since it grows on the ground and is found mainly where it has plant deacaying debris, such as litterfall, in abundance (CORTEZ; BASEIA; SILVEIRA, 2008; PEREIRA, 2010). The species was originally described by Elias Magnus Fries, in 1829, based on material from Brazil (FRIES, 1829; PEREIRA, 2010). *Geastrum lageniforme* Vittad. is the species most related to *G. saccatum*, due to both species having sessile endoperidium, saccharine basidiomata, delimited and fibrillous peristomium, however, *G. saccatum* does not have fibulated hyphae fixed in the outer mycelial layer, a characteristic present in *G. lageniforme* (SUNHEDE, 1989; TRIERVEILER-PEREIRA; CALONGE; BASEIA, 2011). *Geastrum saccatum* produces non-mature basidiomes in which the apical fragment presents pointed characteristics and, after the dehiscence, the exoperidium differentiates into

saculiform, manifesting triangular radium, persistent and smooth mycelial layer, with a bounded and fibrillous peristome (BASEIA; SILVA; CRUZ, 2014).

In morphological analysis, and the following description follows the color code proposed by Kornerup & Wanscher (1978), the species presents expanded and withdrawn basidiome, measuring between 3,9-5,0 cm in diameter × 0,8-2,1 cm in height. Exoperidium opening in 6-8 radiums. Mycelial layer grayish yellow (1B3) in color, formed by unbranched and septate hyphae, with grey (7B1) coloration under optical microscope, measuring between 2-10 µm in diameter. Fibrous layer with orange grey (6B2) coloration, formed by unbranched hyphae with an yellowish white (4A2) coloration under optical microscope, measuring between 2-3 µm in diameter. Pseudoparenchymatous layer with orange grey (6B2) pigmentation, formed by modified globular hyphae, with yellowish white (4A2) coloration under optical microscope, measuring between 17-44 × 10-29 µm. Endoperidium is greenish grey (30B2), with depressed-globose morphology with 0,7-1,6 cm in diameter, and 0,5-1,5 cm in height, peristomium with 0,5 cm in height with grey (4F1) pigmentation in comparison to the endoperidium. The capillary hyphae are erect, with amorphous mass adhered, pale (3A3) to dull yellow (3B3) in color, measuring between 2-6 m in diameter. Basidiospores globose, ornamented with anastomized columns, measuring between 4-5,5 µm in diameter, with a light brown (5D4) coloration under optical microscope. Presence of crystals in the gleba (TRIERVEILER-PEREIRA; CALONGE; BASEIA, 2011).

The species exhibits healing properties for diseases such as asthma and eye infections. Its basidiomes have carbohydrates, lipids and proteins, in which there is a large amount of polysaccharide composed of glucan, which has anti-inflammatory and antioxidant properties (DORE *et al*., 2007). Calcium oxalate crystals were observed in the peridium, in which corroded crystals occur on the outer surface of the endoperidium in mature basidiomes. The calcium oxalate crystals develop before the basidiomata maturation and still remain coupled to the hyphae. These crystals may be associated with the separation system of the exoperidium and endoperidium, during the maturation of the basidiomata (WHITNEY; ARNOTT, 1986).

Based on already reported properties of secondary metabolism compounds of *G. saccatum* in fighting infections and diseases, herein we aimed to evaluate the possible inhibitory potential of the ethanolic extract of this gasteroid fungus on the growth of different *Candida* species strains (*C. albicans*, *C. tropicalis*, and *C. parapsilosis*), potentially pathological for the human being, as a way to investigate and screening new compounds with the potential to be used for the production of antifungal to fight resistant *Candida* strains.

1 Materials and Methods

1.1 *Geastrum saccatum* samples collection

During the months of October, November and December, 2018, mature and non-mature basidiomes of the species *Geastrum saccatum* Fr. were collected in the Environmental Preservation Area – Tucano Ecological Trail (16°17'39" S, 48°56'20" W), located at the Evangelical University of Goiás - UniEVANGÉLICA, in the municipality of Anápolis, Goiás, Brazil. Basidiomes were collected in a area (16°29'35" S, 48°93'79" W) where a great abundance was observed compared to other species in other regions of the Tucano Ecological Trail. For the sampling, collection materials (metric scale, kraft bags, GPS, camera and knife) were used following the fungal material collection literature (GIMENES; MATHEUS, 2010).

Non-mature and mature basidiomes were sampled (Figure 1) and placed in kraft bags for the drying of the material, which remained in an airy environment and under shade for 10 days. The mature basidiomes had on average between 1 and 3 cm of length, being found species with less than 1 cm. Non-mature basidiomes were between 1 and 2,5 cm long.

Figure 1 - Stages of the *G. saccatum* maturation process. (A and B) Non-mature basidiomes. (C) Expanded basidiome.
Font: AUTHORS (2018).

1.2 Obtaining *Geastrum saccatum* extracts

The extracts used in this experiment were obtained through the basidiomes of *G. saccatum*, that were cleaned through the assistance of sieves (MOURA, 2017). Subsequently, the samples were intended for drying the material at room temperature. Then, the material was sprayed with the aid of mortar and pestleand stored in a dark bottle containing ethanol (ratio 1:4) and refrigerated. Finally, the samples were filtered and dried with the aid of rotaevaporator (MORAES *et al*., 2010). The filtration occurred by adding 100 mL of 99% alcohol to the pulverized and filtered sample the next day.

With the obtaining of liquid from the sample, the process continued for three consecutive days at the same time. The extract obtained was packaged in an amber bottle under the cover of light, at 4 ºC (COSTA *et al*., 2013).

1.3 Cultivation and maintenance of *Candida* spp. strains

To carry out the cultivation, strains of *C. parapsilosis* ATCC 22019, *C. albicans* ATCC 18804, and *C. tropicalis* ATCC 90874 were cultivated in Sabouraud Agar Medium Dextrose (peptone 10g/L; dextrose 40g/L; agar 15g/L), kept in incubatorat 36 ºC for seven days, and subjected to experimentation or new repique (MENEZES *et al*., 2012).

1.4 Dilution test in broth

For the inhibition test, the macrodilution method was used according to SNCCLS M27-A2 (NCCLS, 2002), with modifications. Cells of *C. parapsilosis*, *C. albicans,* and *C. tropicalis* were kept in nutrient agar medium supplemented with glucose for seven days at 36 °C, and subsequently inoculated in liquid nutrient medium plus the different concentrations of *G. saccatum* extract (0,5%, 1% and 2%). Serial dilutions of the stock solutions of the extract were arranged in nutrient medium as diluent to achieve final concentrations different from the compounds under study. Spectrophotometric analysis was performed at 520 nm after seven days of growth, when it was possible to determine the minimum inhibitory concentration (MIC) (PRADO *et al*., 2014).

1.5 Sensitivity test on plates

For the sensitivity test in solid medium, samples containing a number of 104 cells of *C. parapsilosis*, *C. albicans* and *C. tropicalis*, with seven days of growth, were added in 27 mL of nutrient agar supplemented with 3 mL glucose, added in different concentrations (0,5%, 1% and 2%) of extract of *G. saccatum*. The plates were incubated for seven days at 36°C, with observations on the third day (BETONI *et al*., 2006).

2 Results and Discussion

2.1 Sensitivity test on plates

To evaluate the influence of *G. saccatum* extract on the growth of *C. albicans*, *C. tropicalis,* and *C. parapsilosis*, a plate sensitivity test was performed. The experiment was conducted in three plates and on the third day the growth rate of *C.*

albicans, *C. tropicalis* and *C. parapsilosis* was observed qualitatively and the photographic record was held. In different concentrations of *G. saccatum* extract added to plates containing *C. albicans*, *C. parapsilosis,* and *C. tropicalis*, it was not possible to observe any inhibition of the growth of the fungi. Fungal growth occurred in all concentrations tested in contrast to the plate containing only the culture medium and fungus (control). It was also observed that the ethanolic extract of *G. saccatum* stimulated colony growth. Thus, it is possible to state that the extract of *G. saccatum* does not inhibit the growth of the fungus at the tested concentrations (Figure 2).

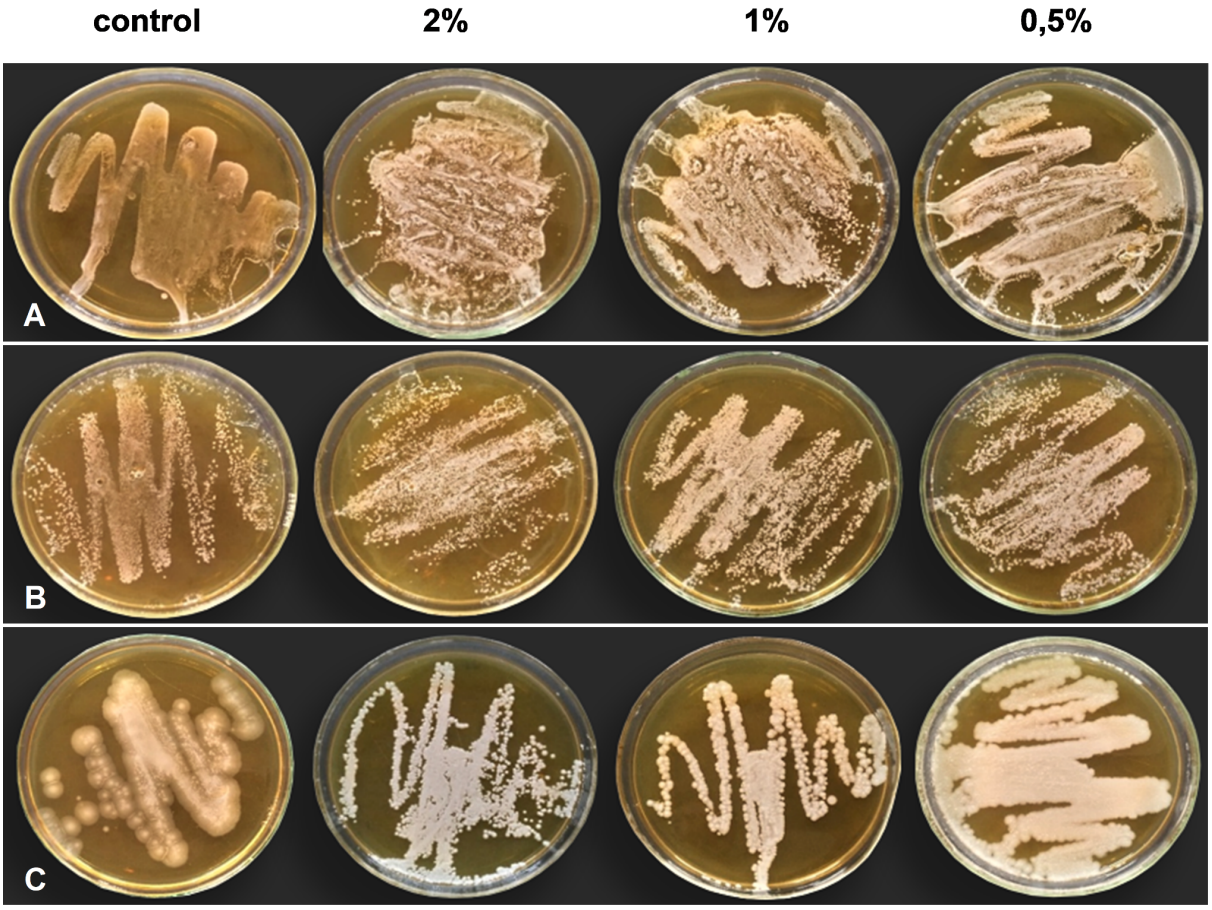

Figure 2 - Growth of different species of *Candida*, in nutrient agar supplemented with ethanolic extract of *G. saccatum* at concentrations of 2%, 1% and 0,5%. (A) *C. albicans*. (B) *C. parapsilosis*. (C) *C. tropicalis*.
Font: AUTHOR (2019).

In an experimental essay, the mushroom species *Lentinula edodes* (Berk.) Pegler (shiitake) has successfully inhibited *C. albicans* strains. On the other hand, the species of mushrooms *Agaricus blazei* Murrill, *A. bonaerensis* Speg., *Pleurotus ostreatus* (Jacq.) P. Kumm., and *Pholiota microspora* (Berk.) Sacc., showed negative results for inhibition (PACCOLA *et al*., 2001), as well as the extract of *G. saccatum*, analyzed in this study. By other hand, the species *A. bisporus* (J.E. Lange) Imbach, *A. bitorquis* (Quél.) Sacc., and *A. essettei* Bon showed potential for antifungal action against strains of *C. albicans* and *C. tropicalis* (ALVES *et al*., 2013).

The ethanolic extract of shiitake mushroom showed stronger antifungal potential against *C. albicans* yeast compared to its aqueous extract. The ethanolic extract was able to significantly reduce the count of *C. albicans* without biofilm formation, with the inhibitory concentration above 25 mg/mL, while the aqueous extract did not show any inhibitory activity (YOO, 2021).

In the evaluation of the inhibitory activity, the crude extract of the species *Irpex lacteus* (Fr.) Fr. was successful in inhibiting the growth of *C. albicans* ATCC 18804 and *C. parapsilosis* ATCC 22019 strains, while the species *Oudemansiella canarii* (Jungh.) Höhn was able to inhibit the action of *C. tropicalis* ATCC 750 and *C. albicans* ATCC 18804 strains. In both strains the anti-candida activity of the species *Agaricus* cf. *nigrecentulus* Heinem., *Agrocybe perfecta* (Rick) Singer, *Donkia pulcherrima* (Berk. & M.A. Curtis) Pilát [=*Climacodon pulcherrimus* (Berk. & M.A. Curtis) Nikol.], *Gloeoporus thelephoroides* (Hook.) G. Cunn., *Hexagonia hydnoides* (Sw.) M. Fidalgo, *Leucoagaricus* cf. *cinereus* (Quél.) Bon & Boiffard, *Marasmius* cf. *bellus* Berk., *Marasmius* sp., *Nothopanus hygrophanus* (Mont.) Singer ex Pegler, *Pycnoporus sanguineus* (L.) Murrill, *Phellinus* sp., and *Trullella duracina* (Pat.) Zmitr. [=*Tyromyces duracinus* (Pat.) Murrill] as there was no inhibition of growth, as we observed in the present study (ROSA *et al*., 2003).

The extra and intracellular fungal extracts of *Penicillium melinii* Thom, *Petriella setifera* (Alf. Schmidt) Curzi, *Aspergillus tubingensis* Mosseray (=*Aspergillus pseudoniger* Mosseray), *Alternaria chlamydospora* Mouch., *Pythium nayoroense* T. Watanabe, *Didymella glomerata* (Corda) Qian Chen & L. Cai [=*Phoma glomerata* (Corda) Wollenw. & Hochapfel], *Mucor ramosissimus* Samouts., *Mucor racemosus* Bull., *Fusarium chlamydosporum* Wollenw. & Reinking, and *Rhizopus microsporus* Tiegh. (=*Rhizopus azygosporus* G.F. Yuan & S.C. Jong) strains, were tested to evaluate the antifungal action capacity against *Candida* strains. The *Didymella glomerata* [=*Phoma glomerata*] extracts did not show any inhibitory activity against *C. albicans*, *C. dubliniensis*, *C. famata* (F.C. Harrison) S.A. Mey. & Yarrow [=*Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij], *C. glabrata*, *C. inconspicua*, *C. kefyr*, *C. krusei*, *C. norvegensis* Dietrichson ex Uden & H.R. Buckley (=*Pichia norvegensis* Leask & Yarrow), *C. parapsilosis*, and *C. tropicalis*. Compensatorily, the extracts of most fungal isolates showed moderate inhibitory activities against most *Candida* species. The *Aspergillus tubingensis* (=*Aspergillus pseudoniger*) extract exhibited good antifungal activity against *C. parapsilosis*, *C. albicans*, and *C. krusei* (AL-ENAZI *et al*., 2018).

Another study with *Geastrum triplex* Jungh, species demonstrates its antibacterial potential to inhibit bacterial pathogens of plants and humans. The study evaluated the species *Agrobacterium tumefaciens* MTCC-431, *Escherichia coli* MTCC-1698, *Xanthomonas campestris* MTCC-2286, *Klebsiella pneumonia* MTCC-7028, *Pseudomonas aeroginosa* MTCC-1934, *Pseudomonas syringae* MTCC-1604, *Salmonella parathyphi* MTCC-1088, *Salmonella typhi* MTCC-968, and

Staphylococcus aureus MTCC-902 in different extracts of *G. triplex* (petroleum ether extract, chloroform extract, and methanol extract). In plant pathogenic bacteria, the petroleum ether extract had a positive result in the inhibition of species *A. tumefaciens* in all tested concentrations (100%, 50%, 25% and 12,5%). With the chloroform extract, positive inhibition occurred in species *X. campestris* in all concentrations. In human pathogenic bacteria, the species *S. parathyphi* did not show inhibition against the methanol extract of *G. triplex*. However, it showed inhibition in the remaining extracts of all concentrations. In the petroleum ether extract, it obtained greater success, mainly in the species *E. coli* and *S. aureus* (inhibition in all concentrations), *S. typhi* in which only the concentration 12,5% was unsuccessful. The chloroform extracts and methanol extract obtained reasonable results in the species *P. aeroginosa*, *S. typhi*, *S. aureus*, *E. coli* and *K. pneumonia* (CHITTARAGI *et al*., 2013).

2.2 Dilution test in broth

The experiment for inhibition was done by the macrodilution method, with the concentrations of 0,5%, 1% and 2% ethanol extract of *G. saccatum*. Analyzing Table 1, it is observed that no inhibition of growth of *Candida* species occurred in any of the tested concentrations, making it impossible to calculate the minimum inhibitory concentration (MIC). This way, the tests reinforce the results of the sensitivity tests on plates.

	MIC
C. albicans	N.F.
C. parapsilosis	N.F.
C. tropicalis	N.F.

*E.E. = Total ethanolic extract
**N.F. = No value found

Table 1 Minimum inhibitory concentration of *Geastrum saccatum* total extract against *Candida albicans*, *C. parapsilosis* and *C. tropicalis*.

The determination of minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) was carried out using the technique of microdilution of the mycelial extract and the crude protein of the medicinal fungus *Ophiocordyceps sobolifera* (Hill ex Watson) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora in contrast with *C. albicans*. The MICs of the mycelial extracts were relatively low, with the mycelial extract Cod-KK1643 showing the most significant activity, with MIC values of 0.78 mg/mL, while the MICs of the crude proteins of all the isolates obtained results more significant than 0,5 mg/mL. The mycelial extracts of Cod-KS1601, Cod-SN1606, and Cod-KK1643 had the lowest MFC value, while the extracts of Cod-NB1302, Cod-

KK1506, Cod-SN1610, and Cod-SN1626 were 50 mg/mL, in crude proteins the MFCs of all isolates were more outstanding than 0,5 mg/mL (SANGDEE et al., 2018).

Different extracts of the *Pleurotus giganteus* (Berk.) S.C. Karunarathna & K.D. Hyde (=*Lentinus giganteus* Berk.) mushroom were used to determine the MIC against *Candida* strains. The aqueous extract had minimal or no inhibitory activity against all *Candida* species tested, as observed to *G. saccatum* extract analyzed in this study. However, the ethyl acetate extract showed an inhibitory action on the growth of all *Candida* strains (*Candida albicans* WM1172, *Candida albicans* ATCC90028, *Candida dubliniensis*, *Candida glabrata* CBS138, *Candida glabrata* ATCC90030, *Candida krusei* ATCC6258, *Candida pseudotropicalis*, *Candida tropicalis* WM30), when tested to 50 and 100 µg/mL. On the other hand, for methanol extract, the strains of *Candida dubliniensis*, *Candida glabrata* CBS138, and *Candida glabrata* ATCC90030, showed sensitivity only at a concentration of 100 µg/mL (PHAN et al., 2013).

In *Geastrum* species, the search for MIC was held by microdilution methodology to analyse its antibacterial action in *E. coli* and *S. aureus*. Tests were performed on different extracts: *Geastrum entomophilum* in the non-mature and mature stages and *Geastrum entomophilum* in the mature stage. The tests performed were negative in different extracts for the strains tested, as were the tests performed in this study with *G. saccatum* in *Candida* species (MOURA, 2017).

The antimicrobial action of different species of the fungus *Pleurotus* against *Bacillus subtilis*, *C. albicans*, *E. coli* and *S. aureus* was verified through disc diffusion test and comparison test. In disc diffusion, the *P. ostreatus* DSM 1833, *P. ostreatus* CCB 001 and *P. sajor-caju* CCB 019 strains did not present inhibitory halo in any of the organisms tested. Positive results occurred only in the *Pleurotus spp*. against *B. subtilis*. In the comparison test, the *P. ostreatus* DSM 1833 and *P. ostreatus* CCB 001 strains obtained reasonable results against *B. subtilis*, *S. aureus* and *C. albicans*. The strain *P. sajor-caju* CCB 019 did not obtain positive results for inhibition of organisms, and the strain *Pleurotus spp* showed inhibition only in the species *B. subtilis* (WISBECK; ROBERT; FURLAN, 2002).

In antimicrobial activity of two extracts (polar and apolar extract) obtained from *Pycnoporus sanguineus* on *E. coli* (ATCC-25923), *S. aureus* (ATCC-25922), *Staphylococcus epidermidis* (ATCC-12228), *Pseudomonas aeruginosa* (ATCC-9027) and *C. albicans* (ATCC-10231), the extracts obtained satisfactory results for inhibition of the organisms tested. In both extracts the strain of *S. aureus* inhibition occurred in the concentrations of 100%, 50%, 25% and 12,50%, in which in the apolar extract inhibition occurred up to the concentration of 6.25%. Within the *P. aeruginosa* strain, inhibition was displayed in both systems, occurring at 100% and 50% concentrations. Meanwhile, in the *S. epidermidis* strain inhibition occurred only with the apolar extract, at concentrations of 100%, 50% and 25%. The strains *E. coli* and *C. albicans* obtained negative results in both extracts (VANDERLINDE; ONOFRE, 2010).

Conclusion

Many studies show that several species of *Candida* are resistant to current antifungal drugs. Thus, the screening for new and efficient antifungal compounds has been pointed, at the same time, as a trending and gap in *Candida*/candidiasis research, due to the scarcity of data, in pharmacological studies. In this context, the present study demonstrated that the ethanol extract of *G. saccatum* does not inhibit the growth of *C. albicans*, *C. parapsilosis* and *C. tropicalis*, but does not exclude the possibility of other types of *G. saccatum* extract being tested in different concentrations against new strains of *Candida*, as well as other microorganisms.

This study made it possible to investigate how two taxonomically closely related organisms (Fungi: Dikarya), but with distinct life histories, can exhibit antagonistic effects on the growth of the other through secondary metabolism unique to the Fungi Kingdom. It is known that there are several fungal species with the potential to fight other fungi and, considering the estimates of richness, studies such as this one should be intensified. Therefore, this study contributes methodological approaches which may possibly further research on these organisms, from different hypotheses based on the same methodological approach, focusing mainly on the native funga from Cerrado.

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