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Evaluation of the antimicrobial activity of the crude ethanol extract, essential oil, and fractions from *Campomanesia pubescens* leaves

Avaliação da atividade antimicrobiana do extrato etanólico bruto, óleo essencial e frações das folhas de *Campomanesia pubescens*

Evaluación de la actividad antimicrobial del extracto de ethanol crudo, aceite esencial y fracciones de hojas de *Campomanesia pubescens*

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Pablo Henrique dos Santos Silva
ORCID: https://orcid.org/0000-0002-3787-2545
Federal University of Goiás, Brazil
E-mail: pabloh.ufg@gmail.com
Fernando Yano Abrão
ORCID: https://orcid.org/0000-0002-0553-0946
Federal University of Goiás, Goiânia, Brazil
Facunicamps, Brazil
E-mail: yanobruce@gmail.com
Jose Realino de Paula
ORCID: https://orcid.org/0000-0002-4424-7692
Federal University of Goiás, Brazil
E-mail: pjrpaula@gmail.com
Heleno Dias Ferreira
ORCID: https://orcid.org/0000-0001-7763-734X
Federal University of Goiás, Brazil
E-mail: hdiasicb@gmail.com
Tatiana de Sousa Fiuza
ORCID: https://orcid.org/0000-0003-0135-177X
Federal University of Goiás, Brazil
E-mail: tatianaanatomia@gmail.com

Abstract
Campomanesia pubescens (DC.) O. Berg (Myrtaceae), widely known as "gabiroba", is a shrub or subshrub used in folk medicine (leaves and barks of the stem) in the form of decoction or infusion in combating urinary tract disorders, diarrhea, and as an astringent. The present work aimed to evaluate the antimicrobial activity of essential oil, crude ethanol extract and hexane fractions, dichloromethane, ethyl acetate, and aqueous from *C. pubescens* leaves against Gram-positive, Gram-negative bacteria, and fungi. The leaves were collected in Hidrolândia - Goiás and the essential oil was obtained by hydrodistillation in a Clevenger apparatus. The extract and fractions were tested against bacteria and fungi using the microdilution method. The aqueous fractions and ethyl acetate showed strong inhibiting activity (MIC= 8 to 16 µg/mL) against *Candida glabrata*, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* and moderate activity (MIC= 32 to 64 µg/mL) against *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*. There is no report in the literature on the antifungal activity of ethanol extract and fractions of the leaves of *C. pubecens*. It is concluded through this study that the aqueous fractions and ethyl acetate from *C. pubecens* leaves presented strong antifungal activity which may justify its popular use in the treatment of urinary tract disorders. Therefore, this species has therapeutic potential that underlies more in-depth studies. This is the first study of the antifungal activity of crude extract and fractions from *C. pubecens* leaves.
Keywords: *Candida glabrata*; *Candida krusei*; *Criptococcus neoformans;* Myrtaceae.

Resumo
Campomanesia pubescens (DC.) O. Berg (Myrtaceae), popularmente conhecida como gabiroba, é um arbusto ou subarbusto utilizado na medicina popular (folhas e cascas do caule) em forma de decocção ou infusão no combate a afecções do aparelho urinário, na diarreia e possui ação adstringente. O presente trabalho teve como objetivos avaliar a atividade antimicrobiana do óleo essencial, extrato etanólico bruto e frações hexano, diclorometano, acetato de etila e aquosa das folhas da *C. pubescens* frente a bactérias Gram positivas, Gram negativas e fungos. As folhas foram coletadas em Hidrolândia – Goiás e o óleo essencial obtido por hidrodestilação em um aparelho de Clevenger. O extrato etanólico bruto e as frações foram testadas contra bactérias e fungos pelo método de microdiluição em poço. As frações aquosa e acetato de etila apresentaram atividade inibitória forte (MIC= 8 a 16 µg/mL) contra *Candida glabrata*,

Criptococcus neoformans e *Sacaromices cerevisae* e atividade moderada (MIC= 32 a 64 µg/mL) contra *Candida krusei*, *Candida parapsilosis* e *Candida tropicalis*. Não há relato na literatura pesquisada sobre a atividade antifúngica do extrato etanólico e frações das folhas de *C. pubecens*. Conclui-se por meio desse estudo que as frações: aquosa e acetato de etila das folhas de *C. pubecens* apresentaram atividade antifúngica forte o que pode justificar seu uso popular no tratamento de afecções do aparelho urinário. Portanto essa espécie possui potencial terapêutico que fundamenta estudos mais aprofundados. Esse é o primeiro estudo da atividade antifúngica do extrato bruto e frações das folhas da *C. pubecens*.
Palavras-chave: *Candida glabrata*; *Candida krusei*; *Criptococcus neoformans;* Myrtaceae.

Resumen
Campomanesia pubescens (DC.) O. Berg (Myrtaceae), conocida popularmente como gabiroba, es un arbusto o subarbusto utilizado en medicina popular (hojas y corteza de tallo) en forma de decocción o infusión para combatir trastornos del tracto urinario, diarrea y tiene acción astringente. El presente trabajo tuvo como objetivo evaluar la actividad antimicrobiana del aceite esencial, extracto etanólico crudo y hexano, diclorometano, acetato de etilo y fracciones acuosas de hojas de *C. pubescens* contra hongos y bacterias Gram positivos y Gram negativos. Las hojas fueron recolectadas en Hidrolândia - Goiás y el aceite esencial obtenido por hidrodestilación en aparato Clevenger. El extracto de etanol crudo y las fracciones se probaron contra bacterias y hongos por el método de microdilución de pozo.Las fracciones acuosa y de acetato de etilo mostraron una fuerte actividad inhibitoria (MIC= 8 a 16 µg/mL) contra *Candida glabrata*, *Cryptococcus neoformans* y *Sacaromyces cerevisae* y actividad moderada (MIC= 32 a 64 µg/mL) contra *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*. No existe reporte en la literatura investigada sobre la actividad antifúngica del extracto etanólico y fracciones de las hojas de *C. pubecens*. Se concluye de este estudio que las fracciones acuosa y de acetato de etilo de las hojas de *C. pubecens* mostraron una fuerte actividad antifúngica, lo que puede justificar su uso popular en el tratamiento de trastornos del tracto urinario. Por lo tanto, esta especie tiene un potencial terapéutico que apoya estudios posteriores. Este es el primer estudio de la actividad antifúngica del extracto crudo y fracciones de hojas de *C. pubecens*.
Palabras clave: *Candida glabrata*; *Candida krusei*; *Criptococcus neoformans;* Myrtaceae.

1. Introduction

Myrtaceae currently contains 131 genera and 5,900 species and is divided into two subfamilies: 1. Psiloxydeae Schimid with only 2 genera and 4 species, being subdivided into two tribes: Heteropyxideae Harvey and Psiloxyleae (Croizat) A. J. Scott; 2. Myrtoideae Sweet with 129 genera and 5,894 species (APG IV, 2016). In Brazil, it is represented by 23 genera and about 1034 species distributed in the Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal (Sobral, et al., 2015). They are distributed in tropical and subtropical regions (APG III, 2009).

Campomanesia Ruiz & Pav. contains about 80 species (APG IV, 2016). It has about 36 species in South America (Govaerts, et al., 2008), of which 31 are present in Brazil (Sobral, et al., 2010). In Goiás there are 5 species *Campomanesia adamantium* (Cambess.) O. Berg, *Campomanesia eugenioides* (Cambess.) D. Legrand, *Campomanesia pabstiana* Mattos & D. Legrand, *Campomanesia sessiliflora* (O. Berg) Mattos, and *Campomanesia pubescens* (Forzza, et al., 2010). This genus is characterized by an ovary 4–18–locular, presenting several eggs per lumocle (Landrum, 1982; Landrum & Kawasaki, 1997). The leaves have opposite phyllotaxis with a shape ranging from oval to elliptical-lanceolate in some species and from oblong to elliptical in others, the venation pattern is camptódromo-brochidodromous and its color varies from light green to dark green. Secretory cavities and trichomes are found throughout the limbus, with a higher incidence in the abaxial face (Saibert, 2016).

Some studies have demonstrated important therapeutic activities, such as anti-ulcerogenic, anti-inflammatory, and antiprotozoal effects, as well as anti-diarrheic and antimicrobial activity, antioxidant potential, and antiplatelet, antithrombotic, fibrinolytic, and, more recently, hypotensive effects in *Campomanesia species* (Cardozo, et al., 2018). Klafke, et al. (2012) verified the antiplatelet, antithrombotic, and fibrinolytic activity of *the* leaf extract of *Campomanesia xanthocarpa*, Santos, et al. (2019) antimicrobial and antioxidant activity of the essential oil of *Campomanesia guazumifolia* (Cambess.) O. Berg, Kuhn, et al. (2019) antibiofilm activity of the essential oil of the leaves of *Campomanesia aurea* O. Berg, Sá, et al. (2018) antimicrobial activity from *Campomanesia adamantium* leaves, Lorençoni, et al. (2020) anti-inflammatory activity of the essential oil from

Campomanesia phaea (O.Berg) Landrum leaves, and Mariott, et al., (2021) antimicrobial and antidiarreic activity of the hydroethanolic extract and dichloromethane and ethyl acetate fractions from *Campomanesia reitziana* D. Legrand leaves.

Campomanesia pubescens (DC.) O. Berg (Myrtaceae), widely known as "gabiroba", is a shrub or subshrub 0.5–1.5 m high. According to Sobral (2015), the leaves are densely covered by trichomes on both sides. It is characterized by producing white and delicate flowers during the flowering period (Almeida, et al., 2000), has bracts in the form of scales or small leaves, acute sepals, rounded or intermediate forms, green or yellow-green fruits, and ovaries with 4 to 7 glasses (Landrum, 1986). The fruits ripen between November and December, the pulp is juicy with acidulate flavor (Lorenzi, et al., 2006), with high content of vitamin C (1000mg/100g) and phenolic compounds (Duarte, et al., 2009). Anatomically, the leaf of *C. pubescens* is hypostomatic with anomocytic stomata, coated with non-branched, unicellular trichomes with no pedal cell on both sides. The presence of idioblasts with polyhedral or druse crystals and secretory cavities (Costa, et al., 2021).

The leaves and barks of the stem of *C. pubescens* are used in folk medicine in the form of decoction or infusion in combating urinary tract disorders and diarrhea and have astringent action (Rodrigues & Carvalho, 2007).

An *in vitro* study was conducted with hexane extract and hexane fractions from *C. pubescens* fruits verified antimicrobial activity against *Staphylococcus aureus* (CIM=15.0 μg/mL), *Pseudomonas aeruginosa* (CIM=15.0 μg/mL), *Escherichia coli* (CIM=20.0 μg/mL), *Salmonella setubal* (CIM=15.0 μg/mL), *Saccharomyces cerevisiae* (CIM=15.0 μ g/mL) and *Candida albicans* (CIM=10.0 μg/mL) (Cardoso, et al., 2010). Rocha (2011) verified the high content of phenolic compounds and proanthocyanidins in leaves, stems, roots, and fruits of *C. pubescens*, with antioxidant activity. Chang, et al. (2011) verified the antimicrobial activity of *C. pubecens* roots essential oil against oral pathogen *Fusobacterium nucleatum* (CIM = 62.5 μg/mL) and essential oils of fruits and leaves against *Bacteroides fragilis* (CIM= 125 μg/mL). Guerrero, et al. (2013) verified the potential anti-inflammatory activity of hydroethanolic extract of *C. pubescens* leaves in male rats at a concentration of 250 and 500 mg/kg. According to Cardozo, et al. (2018) the essential oil of *C. pubescens* leaves showed antioxidant capacity and antitumor activity *in vitro* with cells of the human tumor lineage in trials of melanoma, breast, prostate, and colon cancer. Pradella, et al. (2021) showed that the ethanol extract from *C. pubescens* leaves was able to accelerate the healing of skin wounds infected by *Staphylococcus aureus* in rats, compared with other topical antimicrobials.

Aiming at pharmacological potential, the present work aims to evaluate the antimicrobial activity of essential oil, crude ethanol extract, and fractions hexane, dichloromethane, ethyl acetate, and aqueous from *Campomanesia pubescens* leaves against Gram-positive, Gram-negative bacteria and fungi.

2. Methodology

The botanical material was collected in Hidrolândia - Goiás (786 m altitude, 16° 53 '59" S and 49 ° 13' 29" W). The botanical identification was performed by Prof. Dr. José Realino de Paula and an exsiccate was deposited in the Herbarium of the Federal University of Goiás (no. 67844). The leaves were dried in an oven with air circulation at 37º C for 3 days.

2.1 Obtaining essential oil

The botanical material consisting of dried leaves of *C. pubescens* was pulverized in a knife mill (Skymsen, LS-08MB-N) and immediately submitted to hydrodistillation in a Clevenger apparatus for two hours to obtain the essential oil. The oils were stored in glass vials at a temperature of -18 °C until further analysis.

2.2 Obtaining the crude ethanol extract and fractions

For the preparation of the crude ethanol extract, the dried leaves were pulverized in a knife mill (Skymsen, LS-08MB-N) and macerated with 80% ethanol in the proportion of 1:5 at room temperature, three times and concentrated in a rotary

evaporator at 38º C. To obtain the fractions, the crude ethanol extract was solubilized in methanol. The resulting mixture was extracted by successive liquid/liquid partitions with hexane, dichloromethane, and ethyl acetate (Ferri, 1996). The fractions were concentrated at room temperature and the aqueous fraction was lyophilized.

2.3 Antimicrobial activity

Antimicrobial activity of essential oil, crude ethanol extract, and fractions of ethyl acetate, dichloromethane, hexane, and aqueous was evaluated by determining the Minimal Inhibitory Concentration (MIC) using the broth serial microdilution test according to the Clinical & Laboratory Standards Institute M7-A10 (CLSI, 2015), M27-A3 (CLSI, 2008) and M38-A2 (CLSI, 2017).

The microorganisms used were standard American Type Culture Collection (ATCC) strains purchased at USP and cultivated at the Natural Products Research Laboratory, Federal University of Goiás (UFG) (Table 1).

Table 1 - Microorganisms used in microdilution tests.

Gram-positive bacteria	Gram-negative bacteria	Fungi
Kocuria rhizophila ATCC 9341	*Escherichia coli* ATCC 25922	*Aspergillus brasiliensis* ATCC 16404
Micrococcus luteus ATCC 10240	*Escherichia coli,* ATCC 8739	*Candida albicans* ATCC 90028
Staphylococcus aureus ATCC 29737	*Klebsiella pneumoniae* ATCC 70063	*Candida glabrata* ATCC 90030
Staphylococcus aureus ATCC 6538	*Pseudomonas aeruginosa* ATCC 27853	*Candida glabrata* ATCC 90050
Staphylococcus aureus ATCC 29213	*Salmonella* spp. ATCC 14028	*Candida krusei* ATCC 34135
	Salmonella spp. ATCC 19430	*Candida krusei* ATCC 6258
		Candida parapsilosis ATCC 96143
		Candida parapsilosis ATCC 96141
		Candida parapsilosis ATCC 22019
		Candida tropicalis ATCC 750
		Cryptococcus gatti ATCC 24065
		Cryptococcus neoformans ATCC 28957
		Cryptococcus neoformans ATCC 90112
		Saccharomyces cerevisae ATCC 9763

Source: Authors.

Essential oil, extract and fractions were solubilized in dimethyl sulfoxide (DMSO) 10% (1% in final volume) (CLSI, 2008) and Tween 80 (0.02% in final volume) (Paula, et al., 2012). A stock solution was subsequently prepared in RPMI-1640 buffered with MOPS pH 7.0. In the first holes of the microtiter plate, 200µL of essential oil solution, extract and fractions were added separately and the remaining 100µL RPMI was added. Serial dilution was performed up to the tenth orifice of each line so that concentrations ranging from 2 to 1024 µg/mL were obtained.

For the preparation of microorganisms, a suspension of fungal isolates was prepared in sterile saline solution (NaCl 0.85%), adjusted to 1 x 106 cells/mL, and then dilutions were performed in Roswell Park Memorial Institute (RPMI) broth 1640, resulting in the final concentration of 10^3 CFU/mL. And for the bacteria, the dilutions were performed in Muller Hinton broth, obtaining a final concentration of 10^5 CFU/mL.

In the microtiter plate containing serial dilution of essential oil, crude ethanol extract, and fractions, 100µL of the inoculum was added to each orifice. Thirty different isolates were evaluated, one on each plate. The penultimate column was used to control the growth of the inoculum, and there was no presence of the test compound. The control of the sterility of the medium, containing only RPMI, was performed in the last column.

The plates were incubated at 35 ± 2 ºC for 48h for *Candida* spp. and *Saccharomyces* spp., 72h for *Cryptococcus* spp. and *Aspergillus* spp., and 24 hours for bacteria.

As control groups were used: DMSO 10% (w/v): DMSO 10% (p / v) (as toxicity control), bacteria and fungi (such as microbial growth control), essential oil, crude ethanol extract, and fractions ethyl acetate, dichloromethane, hexane, and aqueous (control of contamination of samples). Ampicillin, fluconazole, *Escherichia coli* ATCC 25922, and *Candida parapsilosis* ATCC 22019 were used as quality control of the technique.

The plates were submitted to visual reading compared to the growth control.

The MIC was defined as the lowest concentration of the sample in µg/mL capable of clearly inhibiting bacterial growth.

According to Alves, et al. (2021) MIC values indicate very strong bioactivity (<3.515 µg/mL), strong (3.516-25 µg/mL), moderate (26-100 µg/mL), weak (101-500 µg/mL), very weak (500-2000 µg/mL) and no bioactivity (> 2000µg/mL).

The determination of CMM was obtained by the broth microdilution test, from which 10 µL of the medium contained in the holes were removed, at concentrations corresponding to MIC, 2x, and 4x and inoculated in a petri dish containing Sabouraud Dextrose Agar (ASD) for fungi and Muller Hinton Agar (MH) for bacteria. After 24 hours of incubation at 35°C for bacteria and 72 hours for fungi, MMC was defined as the lowest concentration that resulted in the growth of fewer than two colonies, representing the death of more than 99% of the original inoculum (Logu, et al., 2005).

All tests were done in triplicate.

3. Results

The yield of the crude ethanol extract was 9.12%, the hexane fraction of 12.04%, the dichloromethane fraction of 34.15%, ethyl acetate fraction of 10.53%, and the aqueous fraction of 5.50%.

3.1 Antimicrobial Activity

The aqueous fraction (FAq) showed strong inhibiting activity against *C. glabrata* ATCC 90030 (16 µg/mL), *C. glabrata* ATCC 90050 (16 µg/mL), *C. gatti* ATCC 24065 (8 µg/mL), *C. neoformans* ATCC 28957 (8 µg/mL), *C. neoformans* ATCC 90112 (16 µg/mL), and *S. cerevisae* ATCC 9763 (16 µg/mL) and moderate activity against *C. krusei* ATCC 34135 (32 µg/mL), *C. krusei* ATCC 6258 (32 µg/mL), *C. parapsilosis* ATCC 96143 (32 µg/mL), *C. parapsilosis* ATCC 96141 (32 µg/mL), *C. parapsilosis* ATCC 22019 (32 µg/mL), and *C. tropicalis* ATCC 750 (64 µg/mL) (Table 2).

The ethyl acetate fraction (FAc) showed strong inhibiting activity against *C. glabrata* ATCC 90030 (16 µg/mL), *C. glabrata* ATCC 90050 (16 µg/mL), *C. gatti* ATCC 24065 (16 µg/mL), C. *neoformans* ATCC 28957 (16 µg/mL), *C. neoformans* ATCC 90112 (16 µg/mL) and *S. cerevisae* ATCC 9763 (16 µg/mL) , moderate activity against *C. krusei* ATCC 34135 (32 µg/mL), *C. krusei* ATCC 6258 (32 µg/mL), *C. parapsilosis* ATCC 96143 (32 µg/mL), *C. parapsilosis* ATCC 96141 (32 µg/mL),

C. parapsilosis ATCC 22019 (32 μg/mL) and weak activity against *C. albicans* ATCC 90028 (128 μg/mL) and *C. tropicalis* ATCC 750 (128 μg/mL) (Table 2).

The crude ethanol extract (EB) showed good inhibiting activity against *C. glabrata* ATCC 90050 (8μg/mL), moderate activity against *C. krusei* ATCC 34135 (32 μg/mL), weak activity against *C. parapsilosis* ATCC 96143 (256 μg/mL), and *C. parapsilosis* ATCC 96141 (128 μg/mL) and very weak activity against *C. tropicalis* ATCC 750 (512 μg/mL) and *C. gatti* ATCC 24065 (512 μg/mL) (Table 2).

The hexane fraction (HF) showed weak activity against *C. glabrata* ATCC 90030 (256 μg/mL), *C. glabrata* ATCC 90050 (256 μg/mL), and *C. gatti* ATCC 24065 (256μg/mL). And very weak injunction activity against *C. neoformans* ATCC 28957 (512 μg/mL) and *S. cerevisiae* ATCC 9763 (512 μg/mL) (Table 2).

The dichloromethane fraction (DF) showed moderate inhibiting activity *against C. glabrata* ATCC 90050 (64 μg/mL), *C. gatti* ATCC 24065 (64 μg/mL), and *S. cerevisiae* ATCC 9763 (64μg/mL), weak activity against *C. neoformans* ATCC 28957 (128μg/mL), and *C. neoformans* ATCC 90112 (256μg/mL) (Table 2).

The aqueous fraction had weak inhibiting activity against *K. rhizophyla* ATCC 9341 (256 μg/mL).

Table 2 - Evaluation of *in vitro* antimicrobial activity of crude ethanol extract, fractions, and essential oil of *Campomanesia pubescens* leaves.

Microorganism	Essential oil CIM (CMM) μg/mL	Crude Ethanol Extract CIM (CMM) μg/mL	Hexane Fraction CIM (CMM) μg/mL	Dichloromethane Fraction CIM (CMM) μg/mL	Ethyl Acetate Fraction CIM (CMM) μg/mL	Aqueous Fraction CIM (CMM) μg/mL
Gram-positive bacteria						
***Kocuria rhizophyla* ATCC 9341**	512 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	512 (512)	256 (512)
***Micrococcus luteus* ATCC 10240**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)
***Staphylococcus aureus* ATCC 29737**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)
***Staphylococcus aureus* ATCC 6538**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)
***Staphylococcus aureus* ATCC 29213**	1024 (>1024)	1024 (>1024)	1024 (>1024)	1024 (>1024)	1024 (>1024)	1024 (>1024)
Gram-negative bacteria						
***Escherichia coli* ATCC 25922**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)
***Escherichia coli* ATCC 8739**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)
***Klebsiella pneumoniae* ATCC 70063**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)
***Pseudomonas aeruginosa* ATCC 27853**	1024 (>1024)	1024 (>1024)	1024 (>1024)	1024 (>1024)	1024 (>1024)	1024 (>1024)
***Salmonella* sp. ATCC 14028**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)

***Salmonella* sp. ATCC 19430**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)
Fungi						
***Aspergillus brasiliensis* ATCC 16404**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)
***Candida albicans* ATCC 90028**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	128 (512)	>1024 (>1024)
***Candida glabrata* ATCC 90030**	>1024 (>1024)	>1024 (>1024)	256 (>1024)	>1024 (>1024)	16 (128)	16 (128)
***Candida glabrata* ATCC 90050**	512 (>1024)	8 (1024)	256 (512)	64 (128)	16 (64)	16 (64)
***Candida krusei* ATCC 34135**	>1024 (>1024)	32 (>1024)	>1024 (>1024)	>1024 (>1024)	32 (1024)	32 (512)
***Candida krusei* ATCC 6258**	>1024 (>1024)	128 (512)	>1024 (>1024)	>1024 (>1024)	32 (128)	32 (64)
***Candida parapsilosis* ATCC 96143**	>1024 (>1024)	256 (>1024)	>1024 (>1024)	>1024 (>1024)	32 (>1024)	32 (256)
***Candida parapsilosis* ATCC 96141**	>1024 (>1024)	128th (>1024)	>1024 (>1024)	512 (>1024)	32 (>1024)	32 (128)
***Candida parapsilosis* ATCC 22019**	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	>1024 (>1024)	32 (1024)	32 (512)
***Candida tropicalis* ATCC 750**	>1024 (>1024)	512 (>1024)	>1024 (>1024)	>1024 (>1024)	128th (>1024)	64 (1024)
***Cryptococcus gattii* ATCC 24065**	>1024 (>1024)	512 (>1024)	256 (256)	64 (256)	16 (32)	8 (32)
***Cryptococcus neoformans* ATCC 28957**	512 (>1024)	1024 (>1024)	512 (>1024)	128th (>1024)	16 (16)	8 (64)
***Cryptococcus neoformans* ATCC 90112**	>1024 (>1024)	1024 (>1024)	>1024 (>1024)	256 (>1024)	16 (32)	16 (64)
***Saccharomyces cerevisiae* ATCC 9763**	>1024 (>1024)	>1024 (>1024)	512 (512)	64 (1024)	16 (64)	16 (32)

Source: Authors.

4. Discussion

The essential oil from *C. pubecens* leaves had weak activity against *K. rhizophyla* ATCC 9341. Chang, et al. (2011) verified weak activity of the essential oil of the leaves of *C. pubescens* collected in Uberlândia - MG against *Bacteroides fragilis* (ATCC 25285) (125 µg/mL) and very weak activity *against Fusobacterium nucleatum* ATCC 25586 (500 µg/mL), *Streptococcus mitis* ATCC 49456 (500 µg/mL) and *Streptococcus mutans* ATCC 25175 (500 µg/mL). From the essential oil of the fruit, weak activity was verified against *B. fragilis* ATCC 25285 (125 µg/mL) and *F. nucleatum* ATCC 25586 (250 µg/mL), and very weak activity against *S. mitis* ATCC 49456 (500 µg/mL) and *Streptococcus mutans* ATCC 25175 (500µg/mL). They also verified weak root essential oil activity against *B. fragilis* ATCC 25285 (250 µg/mL).

The aqueous fractions (FAq) and ethyl acetate (FAc) (MIC= 8 to 16 µg/mL) showed strong inhibiting activity against *C. glabrata*, *C. neoformans,* and *S. cerevisiae* and moderate activity (MIC= 32 to 64 µg/mL) against *C. krusei*, *C. parapsilosis*.

There is no study in the researched literature of the antifungal activity from *C. pubecens* leaves, but there are other species of the same genus. Sá, et al. (2018) verified the strong activity of crude ethanol extract of *Campomanesia adamantium* leaves against *C. neoformans* var. *neoformans* (clinical isolate) L2 (15.62 μg/mL) and *C. neoformans* var. *gatti* (clinical isolate) L1 (15.62 μg/mL), the aqueous fraction *against C. krusei* ATCC 34135 (7.81 μg/mL), *C. tropicalis* ATCC 28707 (7.81 μg/mL) and the ethyl acetate fraction against *C. tropicalis* ATCC 28707 (7.81 μg/mL), *C. neoformans* var. neoformans (clinical isolate) L2 (15.62μg/mL) and *C. neoformans* var. *gatti* (clinical isolate) L1 (15.62 μg/mL). Desoti et al. (2011) observed good antifungal activity from the methanol, hexane, and ethyl acetate extracts of *Campomanesia xanthocarpa* leaves against *C. albicans* and Moura-Costa et al. (2012) reported antifungal activity against three species of *Candida* from *Campomanesia eugenioides* (Cambess.) D. Legrand ex L. R. leaves extracts.

According to Lu, et al. (2019) *C. neoformans* is responsible for fatal lung infections in patients with Cushing's disease in China. In Africa, Hurtado, et al. (2019) observed that cryptococcosis caused by *C. neoformans* and *C. gattii* was the major cause of death in adults with HIV, just as Zamora (2018) found double infection in the central nervous system caused by *Cryptococcus* ssp. patients with HIV. Colombo and Guimarães (2007) found that the microorganisms most commonly responsible for urinary infections were *C. albicans* with approximately 35.5% to 70%, *C. glabrata* with 5% to 33%, and *C. tropicalis* with about 8 to 28% of cases, with a higher frequency of cases in patients who had some exposure to risk factors, such as surveys, hospitalizations and use of a urinary catheter.

According to Tan, et al. (2020) fungal infections are difficult to treat, especially those caused by *Candida* non-*albicans* (*C. krusei*, and *C. tropicalis*) and *C. glabrata* fluconazole-resistant organisms. This difficulty is related to the challenge of achieving an adequate concentration that affects the urinary tract since current agents have low urinary excretion. Therefore, given the strong activity against *Candida and Cryptococcus species*, *C. pubecens* is a promising species for the development of an antifungal drug of natural origin, stimulating further studies.

5. Conclusion

It is concluded through this study that the aqueous fractions and ethyl acetate from *C. pubecens* leaves presented strong antifungal activity which may justify its popular use in the treatment of urinary tract disorders. Therefore, this species has therapeutic potential that underlies more in-depth studies. This is the first study of the antifungal activity of crude extract and fractions of the leaves of *C. pubecens*.

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Declaration of interest

No potential conflict of interest was reported by the authors.

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