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ORIGINAL PAPER

Phytochemical characterization and inhibition of *Candida* sp. by the essential oil of *Baccharis trimera* (Less.) DC

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Abstract

This study aimed to investigate the chemical composition and antifungal potential of the essential oil of *Baccharis trimera* (Less.) DC. against *Candida* strains. The half maximal inhibitory concentration (IC_{50}) was assessed by the microdilution method using the essential oil at a concentration range of 8192 to 8 µg/mL. The minimum fungicide concentration (MFC) was determined by subculture in solid medium. The ability of the essential oil to modulate the activity of antifungals was determined in wells treated simultaneously with the oil at a subinhibitory concentration (MFC/16) and fluconazole (FCZ). The fungal morphology was analyzed by microscopy. Gas chromatography coupled with mass spectrometry (GC/MS) was used to identify the chemical composition. The essential oil presented an CI_{50} of 11.24 and 1.45 µg/mL, which was found to potentiate the effect of FCZ against *Candida albicans*. On the other hand, this combined treatment resulted in antagonism against *Candida tropicalis* and no evident modulation against *Candida krusei* was observed. The essential oil significantly inhibited hyphae growth. However, with a MFC ≥ 16,384 µg/mL, it is assumed that it has a fungistatic action. The antifungal properties demonstrated in this study might be related to the presence of sesquiterpenes and monoterpenes, and the interaction between them. In conclusion, *Baccharis trimera* showed promising anti-*Candida* effects, in addition to potentiating the activity of FCZ against *Candida albicans*, affecting its morphological transition. Therefore, this species constitutes a source of chemical compounds with the potential to be used in the combat of fungal infections.

Keywords *Baccharis trimera* · Terpenes · Essential oils · Anti-*Candida* effect

Introduction

Under specific conditions, some microorganisms of the human microbiota can act as opportunistic pathogens, causing diseases mainly in immunocompromised patients. Evidence has shown that risk factors such as malnutrition, alcohol abuse, and chronic use of immunosuppressive drugs significantly the proliferation of opportunistic pathogens, often leading to severe disease (Sidrim and Rocha 2010). In this context, epidemiological data have indicated that invasive infections by opportunistic fungi represent a significant public health problem with increasing incidence and demonstrated resistance to therapeutic agents, which points to the need of developing new antimicrobials (Mccarthy et al. 2017).

Candida species are notable etiological agents of clinically important fungal infections. Most species in this genus present various virulence mechanisms, including the ability to adhere to human tissues, produce biofilm and secrete toxins (Sardi et al. 2013). Additionally, they can change their morphology (from the yeast-form) by emitting hyphae and pseudohyphae, which increase their pathogenicity since these filamentous

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structures increase their ability to penetrate human tissues (Lu et al. 2014).

The drugs currently used in the treatment of fungal infections present significant toxicity. Moreover, the development of resistance to most commercial antifungal agents has limited the effectiveness of antifungal therapy, especially in immunocompromised patients. Therefore, the investigation of new therapeutic agents capable of directly combating fungal infection or potentiating the action of conventional drugs can represent a cornerstone strategy for the treatment of systemic fungal infections (Maubon et al. 2014; Sardi et al. 2013).

Brazil is known for its rich biodiversity, characterized by a variety of plant species of biological interest. Consistent evidence has demonstrated that these species constitute important sources of chemical compounds with the potential to be used in drug development (Zappi et al. 2015; Newman, 2017). Accordingly, due to the significant ethnobiological importance, many species used in Brazilian traditional medicine were included in the National List of Medicinal Plants of Interest to the Unified Health System—RENISUS (Brasil 2009).

Essential oils are complex volatile compounds extracted from a great variety of aromatic plants. These naturally occurring substances are composed of a mixture of various chemical phytoconstituents produced by the secondary metabolism of plants. Previous research has demonstrated that essential oils exert numerous pharmacological effects, such as antimicrobial, analgesic, sedative, and anti-inflammatory, corroborating the use of aromatic plants in traditional medicine (Simões et al. 2017; Sharifi-Rad et al. 2017).

Baccharis trimera (Less.) DC., popularly known as carqueja, is an herbaceous plant native to Brazil with notable pharmacological importance, and some evidence has indicated that this species has the potential to be used in the production of therapeutic agents (Abad and Bermejo 2007; Karam et al. 2013). Importantly, ethnomedicinal data demonstrated its relevant use in the treatment of gastrointestinal infections (Begossi et al. 2002; Teixeira et al. 2016), suggesting a potential action against invasive abdominal candidiasis (Kullberg and Arendrup, 2015).

Therefore, considering the need to search for new antifungal agents and based on ethnopharmacological studies indicating the pharmacological potential of *Baccharis trimera,* this study aimed to characterize the chemical composition and investigate the antifungal properties of its essential oil against *Candida* strains.

Materials and methods

Extraction and phytochemical analysis of the essential oil

Fresh leaves of *Baccharis trimera* were collected in the Private Reserve of Natural Patrimony (RPPN), a segment of Atlantic Forest in the State of Paraná (Coordinates: S 25° 20.503′, W 049° 48.036′). The collection and transportation of plant material was authorized by the Environmental Institute of Paraná (license number 284/11) The dried specimens were herborized and deposited in the Herbarium of the Federal University of Paraná (registry number 8,257) and duplicates were sent to the Herbarium of the Municipal Botanical Museum of Curitiba (MBM). The use of *B. trimera* in the present study was registered in the National System of Genetic Heritage Management and Associated Traditional Knowledge (SisGen, protocol AC74344).

The leaves of *B. trimera* were dried with an electric dryer (FANEM-320 SE) at 40 °C for 24 h. The essential oil was extracted by hydrodistillation in a Clevenger apparatus. Briefly, 50 g of dry material was subjected to three extraction cycles in a volume of 1 L of distilled water for 4 h (Wasicky 1996). After extraction, the sample was centrifuged at 5000 rpm for 2 min and the oil was collected and stored at − 20 °C, protected from the light.

For chemical analysis, 1.0 µL of essential oil diluted in 1% dichloromethane and injected in an Agilent 6890 chromatograph (Palo Alto, CA) coupled to a selective mass detector Agilent 5973 N with a flow division of 1:20 and injector temperature of 250 °C. The separation of the constituents was obtained using a capillary column HP-5MS (5%-phenyl-95%-dimethylpolysiloxane, 30 m × 0.25 mm × 0.25 µm) with helium as a carrier gas (1.0 mL min^{-1}). The oven temperature was programmed to range from 60 to 240 °C at a rate of 3 °C min^{-1}, with mass detector in electronic ionization mode (70 eV) at 3.15 min-1 $scans^{-1}$ and mass range of 40–450 µ. The transfer line, ion source analyzer (quadrupole), was set at 260, 230 and 150 °C, respectively.

For quantification, the diluted sample was injected into an Agilent 7890A chromatograph equipped with flame ionization detector (FID), operated at 280 ºC using the same column and analytical conditions described above, except for the carrier gas, which was hydrogen (1.5 mL min^{-1}). The relative composition was obtained through the electronic integration of the FID signal, dividing the area of each component by the total area, and expressed as percentage. The chemical constituents were identified by gas chromatography coupled to the mass spectrometer (GC/MS). The mass spectrum and linear retention index of

each constituent was compared to those reported in the literature (Adams 2007) and also with their linear retention indices, calculated from the injection of a homologous series of hydrocarbons (C7–C26) and compared with literature data. The peak analysis was done in the Software GC/MS Postrun Analysis.

The variances were tested for homogeneity using the Bartlett's test and means were compared by the Scott-Knott test using the statistical program ASSISTAT version 7.6 Beta (Silva 2011). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using the *Statistica software* version 8.0. Ward's rule and Euclidean distance were used to compare dendrogram samples (Kaiser 1958; Granato et al. 2012).

Microbiological analysis

Strains and culture media

Standard strains of *Candida albicans* (CAINCQS 40006), *Candida tropicalis* (CT INCQS 40042) and *Candida krusei* (CK INCQS 40095) were obtained from the Oswaldo Cruz Culture Collection (FIOCRUZ) of the Brazilian Institute of Quality Control in Health (INCQS). The clinical isolates CA URM 4127 and CT URM 4262 were provided by the University Recife Mycology (URM) of the Federal University of Pernambuco. These strains were incubated in *Sabouraud dextrose agar* (SDA, KASVI) at 37 °C for 24 h. Then, a sample of each culture was transferred to a test tube containing 4 mL of sterile saline. A standard inoculum was obtained by adjusting the fungal suspension turbidity to a McFarland standard of 0.5 (NCCLS 2002). Double concentrated *Sabouraud Dextrose Broth* (SDB, HIMEDIA), was used in the microdilution assays, while depleted potato dextrose agar (PDA) supplemented with bacteriological agar was used for morphological analysis (Morais-Braga et al. 2016b).

Drugs

The essential oil and fluconazole (Capsule, Prati donaduzzi) were dissolved in dimethyl sulfoxide (DMSO, Merck, Darmstadt, Germany) and dissolved in distilled water. To this end, 0.15 g of each substance was dissolved with 1 mL of DMSO, followed by dilution in distilled water to a concentration of 16,384 µg/mL. As negative control was used the culture medium, distilled water and DMSO. Of note, the final concentration of DMSO does not affect the cell viability (Stoppa et al. 2009).

Determination of the half maximal inhibitory concentration (IC_{50}) and analysis of cell viability

Each well on a 96-well plate was filled with 100 µL of SDB containing 10% of fungal inoculum. Then, 100 µL of essential oil or fluconazole was added to the first well and serially diluted to concentrations ranging from 8192 to 8 µg/mL. The last well (without treatment) was used as control (Javadpour et al. 1996). The plates were incubated at 37 °C for 24 h and then the readings were performed at 450 nm using a spectrophotometer (Thermoplate®). The results obtained were used to construct the viability curve and to calculate IC_{50} of the essential oil of *B. trimera* (Morais-Braga et al. 2016b).

Minimum fungicide concentration (MFC) determination

A sterile rod was used to homogenize the content of each well of a 96-well plate prepared as previously described. Then, it was used to perform a subculture in a Petri dish containing Saboraud dextrose agar, with the aid of a guide card fixed from the bottom of the plate. After an incubation period of 24 h, the formation of *Candida* colonies was analyzed (Ernst et al. 1999; Morais-Braga et al. 2016a). The minimum fungicidal concentration (MFC) was defined as the lowest concentration in which no growth of fungal colonies was observed.

Evaluation of the antifungal enhancing activity in association with fluconazole

The ability of *B. trimera* to potentiate the antifungal activity of fluconazole was assessed as described by Coutinho et al. (2008), with modifications. To this end, the MFC of fluconazole was determined in the presence or absence of the essential oil at a concentration equivalent to its MFC/16. The plates were filled with 100 µL inoculum in medium containing the essential oil. Then, 100 µL of fluconazole was added to the wells at concentrations ranging from 8192 to 8 µg/mL. The plates were incubated at 37 °C for 24 h and then, the readings were performed as previously described (Morais-Braga et al. 2016a).

Morphological analysis

The effects of the essential oil on fungal morphology the development of hyphae were analyzed by optical microscopy in chambers containing sterile slides for the observation of yeasts. Each chamber was filled with 2 mL of sterile distilled water and 3 mL of depleted Potato Dextrose Agar (PDA)-containing oil at four concentrations (MFC/4, MFC/16 and MFC/32). After solidification of the medium, aliquots of the subcultures were used to make two parallel streaks in the solid medium, which was later covered with a sterile

coverslip. The chambers were then placed in the oven at 37 °C for 24 h, and the images were recorded under optical microscope (AXIO IMAGER M2-3525001980- ZEISS-Germany) using a 20× lens. The presence of hyphae and the length of filament extensions was analyzed using the Zen 2.0 software (Carneiro et al. 2019). The experiments were carried out as previously described (Sidrin and Rocha 2010; Morais-Braga et al. (2016b); ; , with some modifications.

Statistical analysis

The data were analyzed for the normal distribution and then, statistical significance was determined using a one-way ANOVA with Bonferroni's post hoc test. The IC_{50} was calculated by a nonlinear regression and expressed as the arithmetic mean ± standard error of the mean. All experiments were performed in quadruplicate and analyzed using Graphpad Prism software version (Carneiro et al. 2019). Statistical significance was considered when $p < 0.05$. The analysis of components is described in the "Extraction and phytochemical analysis of the essential oil" Section.

Results

Chemical constituents of *Bacchais trimera* essential oil

The extraction of the essential oil presented a yield of 0.3% (relative density (d_{20}^{20}): 0.9149 ± 0.0021). By using GC-FID we have obtained the chromatogram of the chemical constituents found in the total oil and the hydrocarbon standard (HC) by using the arithmetic retention index (RI). Figure 1 shows the chromatogram obtained from the essential oil of *Baccharis trimera*. Of the peaks that were generated and the AI values calculated, the constituents were characterized, which total 95.85% of the area. Phytochemical analysis by gas chromatography coupled with mass spectrometry (GC/MS) identified 16 compounds, including carquejyl acetate (28.21%), palustrol (11.53%), β-pinene (9.67%)***** and viridiflorol (7.33%) as major constituents (Table 1 and

Table 1 Chemical composition of essential oil of leaves of *Baccharis trimera*

Constituent	RI_{Cal}	%
Sabinene	972	0.62
β-pinene	976	9.67
(E)-beta-ocimene	1046	1.44
β-elemene	1390	3.94
Carquejyl acetate	1300	28.21
(E)-beta-caryophyllene	1416	4.38
γ-muurolene	1477	3.72
Germacrene D	1479	1.41
Bicyclogermacrene	1493	6.04
δ-cadinene	1522	3.61
Palustrol	1563	11.53
Viridiflorol	1587	7.33
Ledol	1596	4.6
Humelene epoxide II	1604	2.99
β-eudesmol	1643	6.36
α-cadinol	1650	4.16
Monoterpene hydrocarbons		16.3
Oxygenated monoterpenes		28.2
Sesquiterpene hydrocarbons		23.1
Oxygenated sesquiterpenes		32.3
Total		100

Fig.1 Chromatographic profile of *Baccharis trimera* essential oil

Fig. 1). According to our analysis, the essential oil is mainly composed of sesquiterpenes. It is worth noting that terpenes are subdivided into four groups based on the values of the calculated Arithmetic Indices (AI), thus we will have the following: monoterpene hydrocarbons up to 1100; oxygenated monoterpenes from 1100 to 1300; sesquiterpene hydrocarbons from 1300 to 1540; and oxygenated sesquiterpenes above 1540. It is also possible to classify the compounds based on their retention time (TR). Thus, we have the following: monoterpene hydrocarbons below 12 min; oxygenated monoterpenes between 12 and 18 min; sesquiterpene hydrocarbons from 18 to 24 min; and oxygenated sesquiterpenes above 24 min.

Antifungal activity

Half maximal inhibitory concentration and cell viability curve

As shown in Fig. 2, the essential oil inhibited the growth of *Candida* strains only when used at the highest concentrations, demonstrating a clinically ineffective antifungal effect (Houghton et al. 2007). On the other hand, despite the demonstrated resistance, fluconazole (standard control) inhibited fungal growth at lower concentrations.

The IC_{50} values (Table 2) demonstrate that the essential oil potentiated the activity of fluconazole against the CA URM 4127 strain. On the other hand, this combined treatment resulted in antagonistic effects against *Candida tropicalis* strains, and no significant effect was obtained against CK INCQS 40,095.

Minimum fungicide concentration (MFC)

The essential oil presented MFC values above 16,384 µg/mL against all strains, except for CA URM 4127 against which the substance presented an MFC of 256 µg/mL. These findings suggest that the essential oil has a fungicidal action only against this strain. Moreover, when the essential oil was associated with fluconazole, a fungicide effect was observed only in cultures of CA URM 4127 (MFC = 8192 µg/mL) and CK INCQS 40095 (MFC = 4096 µg/mL).

Effects of Baccharis trimera essential oil and fluconazole on fungal morphology

The in vitro treatment with the essential oil of *Baccharis trimera* significantly inhibited morphological changes (hyphae and/or pseudohyphae) in CA INCQS 40,006 (Fig. 3a) MFC/4, MFC/16 and MFC/32. The morphological transition of CA URM 4127 was inhibited by the essential oil at all tested concentrations. In the tests with this strain, fluconazole at MFC/4 (4096 µg/mL) totally inhibited the emission of filaments (not shown).

In CT INCQS 40,042 cultures (Fig. 3b), treatment with both the essential oil and fluconazole at the highest concentration (MFC/4) caused expressive inhibition of morphological changes (hyphae and/or pseudohyphae). However, at lower concentrations (MFC/16 and MFC/32) only the standard drug completely inhibited this phenomenon. The treatment with the essential oil at MFC/4 and MFC/16 caused total inhibition of morphological changes in CT URM 4262 (Fig. 3c), while in tests with CK INCQS 40,095 (Fig. 3d), maximum inhibition of 30% of pseudohyphae was observed, with the highest concentration (MFC/4) of *Baccharis trimera* essential oil. Importantly, the pharmacological control fluconazole inhibited morphological changes at all tested concentrations.

The best result of the antifungal assay with *B. trimera* was the verification of the effect on the morphological transition, suggesting that the essential oil of the study in question has a relevant effect on one of the factors associated with fungal virulence.

Discussion

The present research characterized the chemical composition of an essential oil obtained from *Bacharis trimera* by hydrodestilation. A study by Paroul et al. (2016) using the same extraction method with the *Baccharis trimera* species collected in the state of Rio Grande do Sul identified only three compounds: β-pinene (23.28%), Germacrene D (6.84%) and Bicyclogermacrene (17.58%). However, these compounds were found in higher amounts. Suzuki et al. (2016) also identified β-pinene as the main compound, while in the study of Lago et al. (2008), the major component found was α-humulene. Unlike the present work, Lago et al. (2008) did not identify carquejyl acetate among the chemical constituents of the essential oil of *Bacharis trimera*, which may be related to the influence of environmental factors on the composition of plants Gobbo-Neto and Lopes (2007).

On the other hand, Amaral et al. (2010) identified carquejyl acetate, palustrol and β-pinene as major constituents of this oil. Simões-Pires et al. (2005), also obtained carquejyl acetate as a major compound, although beta-pinene and ledol were also quantified in the sample. A study of Xavier et al. (2011) analyzing the essential oil of three species of *Baccharis* indicated that they had higher yields in winter than in autumn. The study identified major compounds such as D-germacrene, caryophyllene oxide, and spathulenol (*B. dentata*), spathulenol, β-caryophyllene, β-selinene (*B. anomala*), α-pinene, β-pinene and spathulenol (*B. uncinella*).

Studies have shown that *Baccharis* species present high concentrations of carquejyl acetate (Simões-Pires et al.

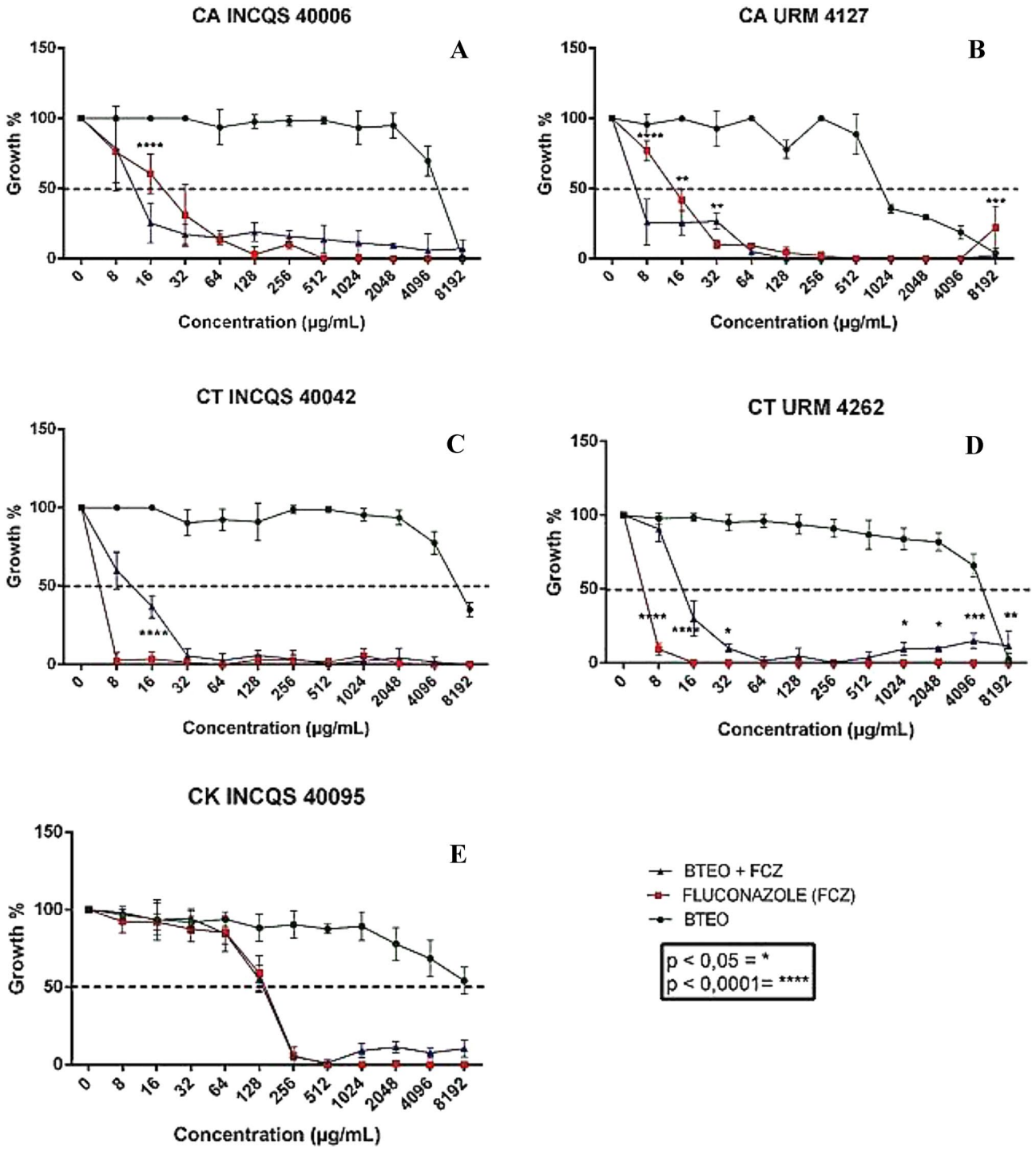

Fig. 2 Effect of *Baccharis trimera* essential oil and fluconazole against *Candida* strains. CA: *Candida albicans;* CT*: Candida tropicalis*; CK: *Candida krusei*; INCQS: Collection Cultures of the National Institute of Quality Control in Health; URM: Universiry Recife Micology; FCZ: Fluconazole; BTEO: *Baccharis trimera* Essential Oil

2005), which has been indicated as a chemotaxonomic marker of *B. trimera essential* oil, corroborating the data obtained in the present analysis. The presence of bioactive compounds such as palustrol and viridiflorol indicates that the importance of this genus from a pharmacological point of view, beyond the consolidated application in the fragrance industry (Minteguiaga et al. 2018). Accordingly, previous research has demonstrated that β-pinene exhibits

Table 2 IC_{50} (50% inhibitory concentration of the fungal population—µg/mL) of the essential oil of *Baccharis trimera* and fluconazole against different strains of *Candida*

	BTEO	FCZ	BTEO + FCZ
CA INCQS 40006	5289.15 µg/mL	18.56 µg/mL	11.24 µg/mL
CA URM 4127	921.15 µg/mL	12.88 µg/mL	1.45 µg/mL
CT INCQS 40042	6529.56 µg/mL	1.58 µg/mL	10.21 µg/mL
CT URM 4262	5433.41 µg/mL	6.15 µg/mL	12.97 µg/mL
CK INCQS 40095	10,990.20 µg/mL	125.06 µg/mL	114.73 µg/mL

CA: *Candida albicans;* CT*: Candida tropicalis*; CK: *Candida krusei*; INCQS: Collection Cultures of the National Institute of Quality Control in Health; URM: Universiry Recife Micology; FCZ: Fluconazole; BTEO: *Baccharis trimera* Essential Oil

insecticidal, pesticidal, insect repellent repellent properties and as such can be used in the control of vectors (Jaenson et al. 2005). On the other hand, viridiflorol was shown to present a moderate in vitro activity against Mycobacterium tuberculosis (Trevisan et al. 2016).

The production of volatile oils in plants is significantly genetically determined. However, it can be influenced by environmental factors such as drought, densities of planting, emission rate, ozone, humidity, CO_2, temperature and light intensity, which in turn can influence photosynthesis, interfering with the production of secondary metabolites (Figueiredo et al. 2008; Rehman et al. 2016). According to Gobbo-Neto and Lopes (2007), other environmental conditions such as rainfall, nutrition and collection time can influence the composition of medicinal plants.

The present antifungal analysis revealed that the essential oil presented clinically ineffective antifungal effect against most *Candida* strains. Nevertheless, studies by other authors in the literature demonstrated that *Baccharis trimera* has antifungal activity against *Colletotrichum* spp., which could be due to the presence of α-cadinol and viridiflorol (Pripdeevech and Chukeatirote, 2010). According to Caneschi et al. (2015), the activity of *Baccharis trimera* essential oil against dermatophytes is attributed to the action of constituents such as β-pinene and carquejyl acetate. This hypothesis is supported by previous research demonstrating that β-pinene had antifungal activity against *Candida* strains (De Macêdo et al. 2018). Simionatto (2004) demonstrated that carquejyl acetate presented weak activity against *Candida albicans* and *Saccharomyces cerevisiae*. A study by Carreira (2007) demonstrated that the essential oil of *B. trimera* presented strong antifungal activity against some species, among which *C. albicans* was the most susceptible to the action of samples obtained during different seasons. This result is similar to that of this study, which had *Candida albicans* as the most susceptible strain.

The antifungal activity of essential oils obtained from other species of *Baccharis* was previously reported. Vannini et al. (2012) demonstrated that *B. uncinella* and *B. semiserrata* inhibited the growth of *Candida albicans* and *C. neoformans* with MIC values of 250 and 500 µg/mL, respectively. The anti-*Candida* activity of *B. dracunculifolia* was demonstrated in a diffusion disc method (Oliveira et al. 2016). On the other hand, a study with an essential oil obtained from the aerial parts of *B. trinervis* found no significant antifungal activity against *Candida albicans, C. tropicalis* and *C. parapsilosis* (Sobrinho et al. 2016).

Essential oil of *Baccharis dracunculifolia* had both fungistatic and fungicidal effect against the *Aspergillus*, *Penicillium* and *Trichoderma* genera, with MIC and MFC values of 8.43 to 16.87 mg/mL (Cazella et al.2019); however, no species of the genus *Candida* has been evaluated. The minimum inhibitory concentration of the essential oil of *B. oreophila* evaluated against *C. albican*s resulted in a MIC of 2500 µg/mL; however, against *C. tropicalis* it did not present activity (Oliveira et al. 2019), and these results are similar to those of this research, where there is a better effect for *C. albicans*.

A study by Dalazen et al. (2011) demonstrated that 93% of *Candida* spp. isolates presented resistance to fluconazole. Favalessa et al. (2010) evaluated the susceptibility of *C. krusei, C. tropicalis* and *C. albicans* against five antifungals and found that most isolates presented significant resistance to fluconazole. Similar to the results of this work, where there is resistance to fluconazole, especially against the strain of *C. krusei* which is naturally resistant to this triazole (Espinel-Ingroff et al. 2014). Here, we showed that the essential oil of *B. trimera* can potentiate the activity of fluconazole against some *Candida* strains, and as such could be useful in the management of infections by resistant fungal strains.

Consistent evidence has indicated that the virulence of *Candida* species is significantly increased by the morphological transition due to antigenic variability. In fact, the development of hyphae contributes to the adhesion and penetration in human epithelial cells, contributing to the disease severity (Santana et al. 2013). This study showed that the essential oil of *B. trimera* significantly inhibited morphological changes in different *Candida* strains.

Although the mechanism underlying this effect remains to be better investigated, evidence suggests that essential oil components may affect fungal morphology by inhibiting enzymatic reactions associated with the cell wall synthesis. Due to their lipophilic nature, terpenoids can cause ruptures in fungal membranes, leading to cell death. Here, it is hypothesized that the antifungal properties demonstrated by the *B. trimera*, whose most promising result was the morphological transition, result from the combined action of monoterpenes and sesquiterpenes, compounds whose antimicrobial activity has been consistently demonstrated (Santos et al. 2010; Mekonnen et al. 2016).

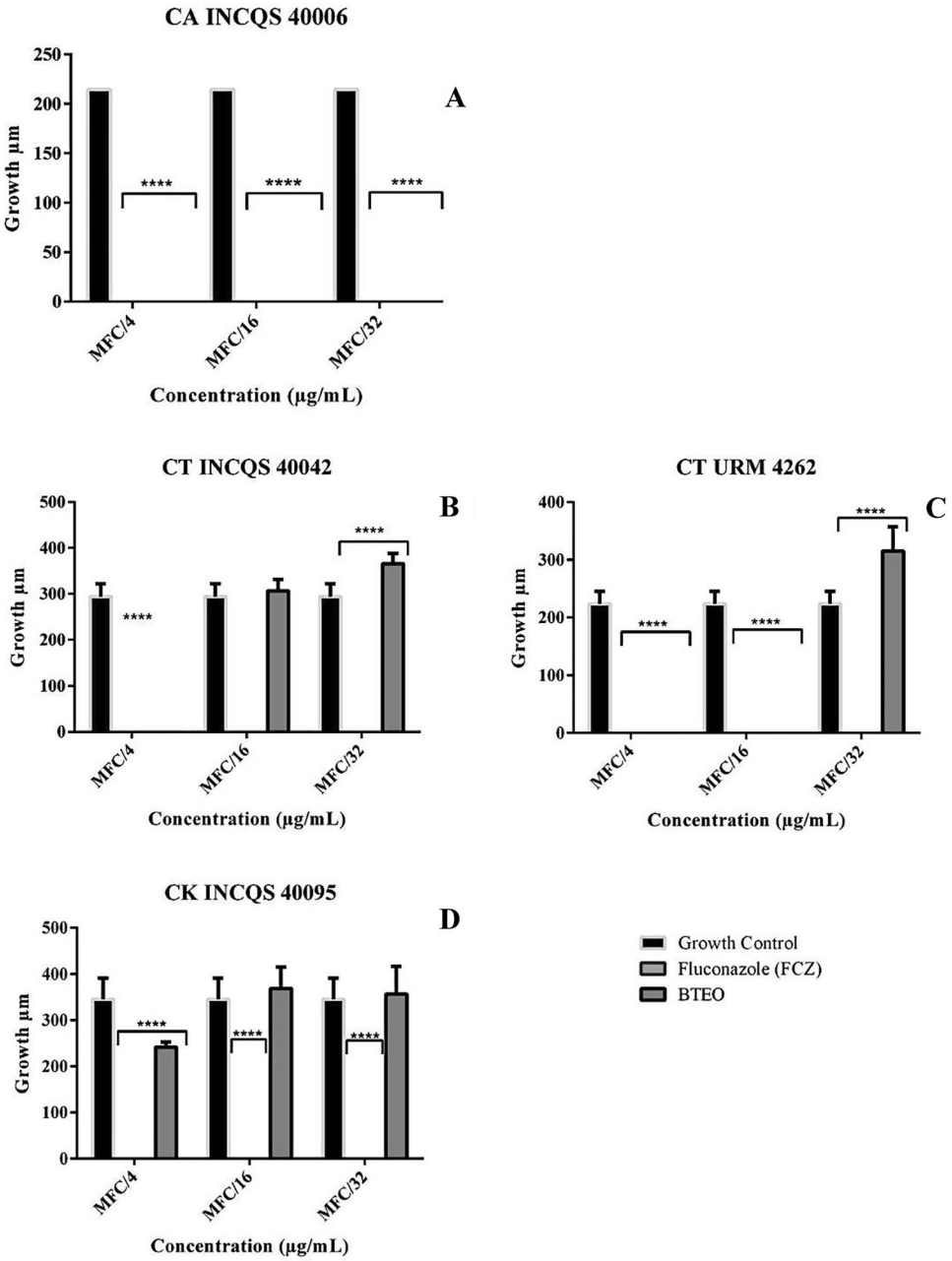

Fig. 3 Effects of *Baccharis trimera* essential oil and fluconazole on fungal morphology. CA: *Candida albicans;* CT*: Candida tropicalis*; CK: *Candida krusei*; INCQS: Collection cultures of the National Institute of Quality Control in Health; URM: Universiry Recife Micology; FCZ: Fluconazole; BTEO: *Baccharis trimera* Essential Oil

Conclusion

The chemical composition of the essential oil of *Baccharis trimera* is characterized by the presence of monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. This substance showed a weak inhibitory effect against *Candida* spp. although it was found to potentiate the effect of fluconazole against *Candida albicans.* Importantly, the essential oil significantly inhibited morphological changes associated increased virulence and pathogenicity, this being the best result. The antifungal properties demonstrated in this study might be related to the presence of sesquiterpenes and monoterpenes, in particular, the major constituent carquejyl acetate.

In conclusion, *Baccharis trimera* showed promising anti-*Candida* effects, in addition to potentiating the activity of fluconazole against *Candida albicans*, affecting its morphological transition. Therefore, this species is a source of chemical compounds with antifungal effects and as such, has the potential to be used in the development of new weapons to combat of candidiasis and other fungal infections.

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Authors' contributions TGS—investigation. JCPS—investigation. JNPC—investigation. WA—validation. CD—validation. JPA—validation. JGMC—methodology. WOA—methodology. LES—investigation. HDMC—methodology. JRF—methodology. MFBM-B—project administration.

Declaration

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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