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Piper regnellii (Miq.) C. DC.: Chemical composition, antimicrobial effects, and modulation of antimicrobial resistance

Ana Lays Braga[a], Rafael Pereira da Cruz[a], Joara Nályda Pereira Carneiro[a], Antonia Thassya Lucas dos Santos[a], Débora Lima Sales[a], Camila Fonseca Bezerra[a], Victor Juno Alencar Fonseca[a], Janaina Esmeraldo Rocha[a], Thiago Sampaio de Freitas[a], Fábia Ferreira Campina[a], Maria do Socorro Costa[a], Wanderlei do Amaral[b], Ricardo Andrade Rebelo[c], Luiz Everson da Silva[b], Jaime Ribeiro-Filho[d], Henrique Douglas Melo Coutinho[a], Fabíola Fernandes Galvão Rodrigues[e], Maria Flaviana Bezerra Morais-Braga[a,*]

[a] *Universidade Regional do Cariri, URCA, Cel Antônio Luis, 1161, 63105-000, Pimenta, Crato, CE, Brazil*
[b] *Universidade Federal do Paraná, UFPR, XV de Novembro, 1299, 80.060-000, Centro, Curitiba, PR, Brazil*
[c] *Universidade Regional de Blumenau, FURB, Antônio da Veiga, 140, 89030-903, Itoupava Seca, Blumenau, SC, Brazil*
[d] *Laboratório de investigação em Genética e Hematologia Translacional, Instituto Gonçalo Moniz (IGM), Fundação Oswaldo Cruz (FIOCRUZ), Salvador, BA 40296-710, Brazil*
[e] *Centro Universitário Doutor Leão Sampaio (Unileão), Av. Maria Letícia Leite Pereira s/n, Lagoa Seca, Juazeiro do Norte, Brazil*

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ABSTRACT

Plant-derived essential oils are volatile hydrophobic compounds with significant antimicrobial activities. Considering the rise of antimicrobial resistance, these natural products have been highlighted as efficient weapons against multidrug-resistant microorganisms. This work aimed to investigate the phytochemical composition and antimicrobial activity of *Piper regnellii* essential oil against strains of *Escherichia coli, Staphylococcus aureus, Candida albicans*, and *Candida tropicalis*. Phytochemical analysis was performed through gas chromatography coupled with mass spectrometry (CG/MS). The intrinsic antimicrobial activity and the ability of the essential oil to modulate antimicrobial resistance were assessed using the broth microdilution method. Fungal virulence inhibition was analyzed by measuring the growth of hyphae in microculture chambers. Phytochemical characterization revealed a predominance of phenylpropanoids, including apiol (70.79%) and dilapiol (15.05%) as major constituents. While presenting clinically ineffective antibacterial effects (MIC $\geq$ 1.024 μg/mL for all strains), the essential oil potentiated the activity of gentamicin against *E. coli* at concentrations above 20 μg/mL. *Piper regnellii* essential oil showed clinically ineffective antifungal activity with IC_{50} values above 500 μg/mL. However, it was found to potentiate the activity of fluconazole against *C. tropicalis* at concentrations ranging from 32 μg/mL to 1024 μg/mL. Furthermore, the morphological transition was inhibited by culturing *C. albicans* and *C. tropicalis* with different concentrations of the essential oil. Together, our results indicate that *P. regnellii* essential oil presents promising antifungal effects. However, the mechanisms underlying its interference on *Candida* virulence remain to be further investigated.

1. Introduction

The widespread increase in infections caused by resistant microorganisms has stimulated the search for new molecules with antimicrobial effects against antibiotic-resistant strains (Silva and Aquino, 2018). In this context, natural product research with an emphasis on the isolation, purification, and characterization of secondary metabolites from medicinal plants has significantly impacted antimicrobial drug discovery, since these secondary metabolites have demonstrated relevant benefits in the treatment of numerous diseases (Zago, 2018; Mulat et al., 2019; Del Quiqui, 2019).

While medicinal plants have been empirically used in the treatment of a variety of infections by low-income populations, evidence has indicated that plant-derived natural products can be successfully employed in the combat of multidrug-resistant (MDR) strains (Rempel et al., 2019). Scientific research has demonstrated that essential oils are effective against pathogenic microorganisms

* Corresponding author.
E-mail address: flavianamoraisb@yahoo.com.br (M.F.B. Morais-Braga).

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(De Almeida, 2020), such as bacteria and fungi. Importantly, increasing evidence indicates that in addition to presenting intrinsic antimicrobial activity, essential oils can potentiate the activity of conventional drugs and as such, are promising substances in the combat of antimicrobial resistance (Santos et al., 2017; Veloso et al., 2017; Janbon et al., 2019).

The genus *Piper* L. (Piperaceae) is composed of species specialized in the production of essential oils. Studies have demonstrated that the therapeutic properties of these species are due to the presence of biologically active metabolites in their essential oils, which have been consistently demonstrated through chemical and pharmacological studies (Pessini et al., 2003; Alves et al., 2016; Rempel et al., 2019; Oliveira, 2020).

Evidence has demonstrated that essential oils obtained from different *Piper* species have the potential to be used in antimicrobial drug development (Bernuci et al., 2016). While the chemical profile of these essential oils has been widely studied (Alves et al., 2016; Bernuci et al., 2016; Mgbeahuruike et al., 2017), the pharmacological profile of isolated constituents remains to be better investigated. Nevertheless, the antibacterial and antifungal activities of some isolated compounds have been previously demonstrated (Salehi, 2019).

The species *Piper regnellii* (Miq.) C. DC., popularly known as "pariparoba, is a shrub used in folk medicine for the treatment of wounds, edema, and skin lesions. In addition to being an ornamental plant, evidence has indicated that this species is pharmacologically active against a variety of pathogens, including Gram-positive and Gram-negative bacteria and against fungi of the genus *Candida* (Pessini, et al., 2003; Salehi, 2019).

Based on this evidence, this study aims to evaluate the chemical composition and antimicrobial activities of *Piper regnellii* essential oil, as well as to investigate its effects on antimicrobial resistance using strains of *Escherichia coli, Staphylococcus aureus, Candida albicans,* and *Candida tropicalis.*

2. Materials and methods

2.1. Botanical material and essential oil extraction

The botanical material was collected in the municipality of Atalanta, Santa Catarina, Brazil (Coordinates: 27° 28' S, 49° 48' W; at 640 m altitude in 09/12/2015). A total of 10 specimens was collected and a voucher specimen was taken to the Herbarium of the Municipal Botanical Museum and registered under registry number MBM085153 (Lawrence, 1951; Instituto Brasileiro de Geografia e Estatística, 1992).

The leaves were dried using an electric dryer (FANEM, Model 320 SE) with air circulation at 40 °C for 24 h. The moisture content of fresh leaves at the time of extraction was determined using 20 g of samples collected in triplicate and dried with air circulation at 65 °C until reaching constant weight.

The essential oil was extracted by hydrodistillation in a graduated Clevenger apparatus. Briefly, 50 g of dried leaves subjected to three cycles of extraction in a glass containing 1L of distilled water at boiling for 2 h (Wasicky, 1963).

2.2. Phytochemical analysis

Phytochemical analysis was performed on a Shimadzu gas chromatograph (model GC-17A) coupled to a mass spectrometer (GC-MS QP5050A) equipped with a fused silica capillary column (J & W Scientific DB-5) with dimensions of 50 mm 0.25 mm × 0.25 μm. Helium was used as a carrier gas at a constant flow of 1.0 mL me-1. The mass spectra were acquired in the range of 40 to 350 Daltons through the method of ionization by electron impact, with ionization energy of 70V and ion source at 200 °C.

The volatile constituents were identified by comparing the mass spectra obtained with the Wiley229 computer library records and by experimentally determining the Kováts indexes, applying a homologous series of n-alkanes under the same conditions used for the injection of essential oils. The values found were compared with the Kováts index. The definitive identification of some volatile constituents was carried out by concomitant injection of chemical standards with essential oils. Only the identifications made based on the mass spectrometry data associated with the co-injection of the oils with the standard analytes were considered definitive.

2.3. Bacterial and fungal strains

Fungal strains were obtained from the Oswaldo Cruz Culture Collection of the National Institute for Quality Control in Health INCQS, and from the Library of the Federal University of Pernambuco (UFPE). Strains CA INCQS 40006 (standard), with access number 10231 and CA URM 4121 (clinical isolate) of *Candida albicans,* and strains CT INCQS 40042 (standard) with access number 13803 and CT URM 4262 (clinical isolate) of *Candida tropicalis* were used for antifungal analysis, while the following standard and resistant strains, obtained from the American Type Culture Collection (ATCC), were used for antibacterial analysis: *Escherichia coli* ATCC (standard) 25922, *Escherichia coli* 06, *Staphylococcus aureus* (standard)ATCC 6538, and *Staphylococcus aureus* 10. The resistance profile of *Escherichia coli* 06 and *Staphylococcus aureus* 10, isolates can be seen in Almeida et al. (2020).

2.4. Inoculum preparation

Fungal strains were cultured in test tubes containing Sabourand Dextrose Agar (SDA - Sigma-Aldrich USA), while the bacterial strains were Heart Infusion Agar (HIA- Sigma-Aldrich USA) for 24 h at 37 °C. The inoculants were prepared in Petri dishes, containing 25 mL of SDA at 37 °C for 24 h. Then, samples were suspended in test tubes containing 0.3 mL of sterile 0.9% saline, and their turbidity assessed according to the McFarland scale (NCCLS, 2002).

2.5. Essential oil preparation

The essential oil was dissolved in 1mL of dimethyl sulfoxide (DMSO - Sigma-Aldrich, USA/ P.A. 99,9%), and then diluted in sterile distilled water to the concentrations of 1024 μg /mL and 4096 μg/mL for antibacterial and antifungal tests, respectively. Of note, the final concentration of DMSO in the samples has no effect on cell viability (Stoppa et al., 2009).

2.6. Standard drugs

The antibiotics norfloxacin, gentamicin, and erythromycin (Cimed - Brazil) were diluted to an initial concentration of 1.024 μg/mL, while fluconazole (Cimed - Brazil), diluted to 4096 μg/mL, was used as a standard antifungal drug. All drugs were diluted in sterile distilled water.

2.7. Minimum inhibitory concentration (MIC) determination

Test tubes were added with 1.350 μL of culture medium and 150 μL of inoculum. A volume of 100 μL of this solution was transferred to wells on a 96-well plate, followed by the addition of the essential oil according to the microdilution method. For antibacterial activity analysis, the essential oil was tested at concentrations ranging from 512 μg/mL to 0.5 μg/mL, while for antibacterial activity analysis, the essential oil was tested at concentrations ranging from 2.048 μg/mL to 0.2 μg/mL. The plates were incubated in the oven at 37 °C for 24 h, after which, 20 μL of resazurin was added to each well and 1 h later, bacterial growth was analyzed by visual observation

(Javadpour et al., 1996). Fungal growth was analyzed by spectrophotometry (Termoplate®), and the readings were used to generate a cell viability curve and determine the IC_{50}. The experiments concerning antibacterial and antifungal activity were performed in triplicate and quadruplicate, respectively. Growth and sterility controls were used in all experiments (morais- Braga et al., 2016).

2.8. Minimum fungicide concentration (MFC) determination

Fungal viability was determined according to the methodology proposed by Ernest et al. (1999) with modifications. The contents of the wells in the plate used to determine the MIC were homogenized with a sterile stem and subcultured in Petri dishes containing SDA (Sabourand Dextrose Agar) culture medium. The plates were incubated at 37 °C and after 24 h, the growth of fungal colonies was determined.

2.9. Analysis of antimicrobial resistance modulation

To analyze the effects of the essential oil on antimicrobial resistance, we followed the method proposed by Coutinho et al. (2008) with adaptations. To this end, standard drugs were combined with subinhibitory concentrations of the essential oil (CIM/8 and CFM/16 for bacterial and fungal cultures, respectively). A solution containing essential oil, culture medium, and inoculum was prepared in test tubes. Then, 100 μL of this solution was added to the wells on a 96-well plate, followed by the addition of 100 μL of the standard drug at different concentrations. The plates were incubated at 37 °C for 24h and the readings were performed as previously described (Morais-Braga et al., 2016).

2.10. Evaluation of fungal virulence modulation

To evaluate the effects of antifungal compounds on fungal virulence, the emission of hyphae by yeasts was analyzed on sterile culture chambers. Briefly, 3 mL of a solution containing culture medium and the essential oil at different concentrations (CFM/2, CFM/4, and CFM/8) were added into humid chambers. Following the solidification medium, two parallel streaks were drawn from the previously prepared inoculum, transferred to the chambers, and covered with a sterile coverslip. The chambers were then taken to the incubator at 37 °C and 24 h later, visualized under optical microscopy (AXIO IMAGER M2-352500198 – ZEISS – Germany) at a 40 X objective. The entire streak was checked for the emission hyphae and photographed with a camera attached to the microscope. The images were analyzed, and hyphae growth was measured using the Zen 2.0 Software (Carneiro et al., 2019). Growth controls were used in all experiments.

2.11. Statistical analysis

The data obtained by spectrophotometry were checked for normal distribution and analyzed by one-way ANOVA followed by Tukey's post hoc test. The MIC values were obtained by non-linear regression through interpolation from standard curves. All analyzes were performed using the Graphpad Prism software version 7.0. Differences with $p < 0.05$ were considered significant.

3. Results

The essential oil extraction showed a yield of 0.85%. The chemical identification was performed using mass spectra compared to data from spectral libraries (Wiley, 1994; NIST, 2016) of linear retention indices calculated from the injection of a homologous series of hydrocarbons (C7-C26) and compared with the literature data (Adams, 2007). As shown in Table 1, the phytochemical analysis identified 94.8% of the constituents, including apiol (70.79%), dilapiol (15.05%), β- eudesmol (2.98%), bicyclogermacrene (2.27%), α-eudesmol (2.14%) and γ-gurjunene (1.25%) as major compounds. With regard to the classes of compounds, it was verified the predominance of phenylpropanoids (85.84%) and sesquiterpenes (8.6%).

Table 1
Chemical composition of the essential oil from the leaves of *Piper regnellii* (Adams, 2007).

Identification Number	Compound	IR /KI	%
1	γ-Gurjunene	14831/477	1.25
2	Bicyclogermacrene	1503/1500	2.27
3	Dilapiol	1636/1620	15.05
4	B-Eudesmol	1661/1650	2.98
5	α-Eudesmol	1664/1653	2.14
6	α-Pineno	932/ 1039	0.50
7	Sapinene	972/1113	1.30
8	Terpinel-4-ol	1174/1181	0.70
9	Aromadandrene	1439/1488	0.40
10	α-Humulene	1452/1455	0.30
11	Cadalene	1655/1678	0.20
12	Germacrene D	1476/1492	0,80
13	Apiol	1695/1678	70.79
Total identified			96.54
Not identified			1.00

Legend: IR – Retention index; KI – Kóvats Index

An analysis of the minimum inhibitory concentration (MIC) demonstrated that the essential oil only caused relevant growth inhibition above 1.024 μg/mL against all strains (not shown), indicating that this natural product has no clinically relevant antibacterial activity (Houghton et al., 2007). In addition, the association of the essential oil with the reference antibiotics norfloxacin and gentamicin against multidrug-resistant strains of *S. aureus* resulted in antagonistic effects, while the effect of erythromycin was not affected. On the other hand, in *E. coli* cultures, the association of oil with gentamicin showed a potentiating effect, causing a reduction in the MIC of this antibiotic. On the other hand, the effect of norfloxacin was antagonized, while the activity of erythromycin was not affected.

Fig. 2 shows the growth curves of *C. albicans* and *C. tropicalis* in the presence of different concentrations of the EOLPR. It was demonstrated that this treatment inhibited fungal growth at the concentrations of 512 μg/mL and 1024 μg/mL for CA URM 4127 and CA INCQS 40006 strains, respectively (Fig. 2 A and B). However, the growth of *C. tropicalis* strains (Fig. 2C and D) was inhibited only when exposed to the highest concentration (2048 μg/mL).

An analysis of the growth curves of CA INCQS 40006 and CT INCQS 40042 revealed no significant difference between the single treatment with the essential oil and the combined treatment with the oil and the standard antifungal drug. About the clinical isolate strains, the association of the oil with fluconazole against CA URM 4127 and CT URM 4262 resulted in antagonism and synergism, respectively.

Table 2 shows the values of half-maximal inhibitory concentration (IC_{50}) for either combined or isolated treatments against different *Candida* strains. The combination of the essential oil with the reference drug resulted in lower IC_{50} values against CA INCQS 40006, CA URM 4127, and CT URM 4262 strains compared to treatment with fluconazole alone. On the other hand, treatment with the standard drug alone was the most effective against CT INQS 40042.

The minimum fungicidal concentration (CFM) was defined as the lowest concentration capable of inhibiting colony growth. The essential oil of *Piper regnellii* showed a CFM of 2048 μg/mL against *C. albicans*, while the CFM for *C. tropicalis* was higher than 4096 μg/mL.

The association of the essential oil with fluconazole resulted in CFM values above 4096 μg/mL against all strains, indicating that this natural product did not enhance the antifungal action of the standard drug. On the contrary, the combination of the substances resulted in antagonism against strains of *C. albicans*.

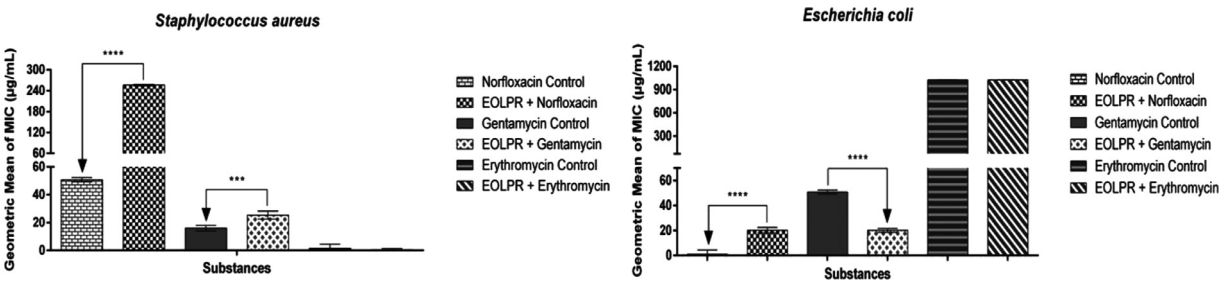

Fig. 1. Antibiotic-modulating activity of the essential oil obtained from the leaves of *Piper regnellii* (EOLPR) in association with norfloxacin, gentamicin, and erythromycin.

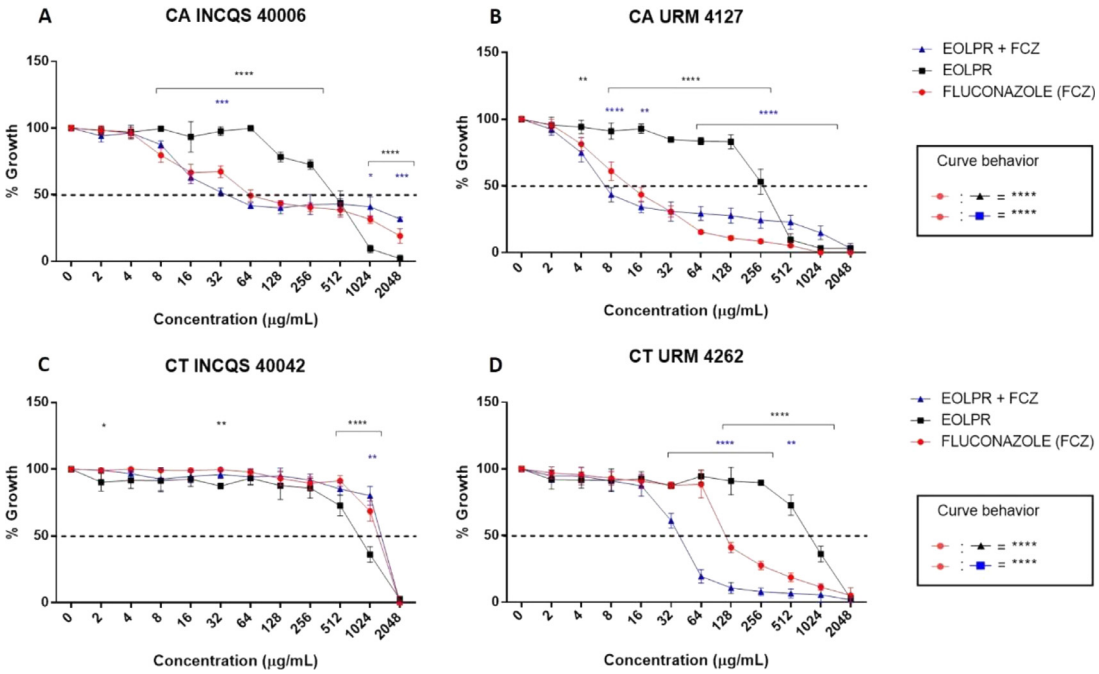

Fig. 2. Antifungal action of fluconazole (FCZ) and EPLPR against *C. albicans* strains CA INCQS 40006 (A) and CA URM 4227 and *C. tropicalis* (CT) strains CT INCQS 40042 (C) and CT URM 4262. INCQS: National Institute for Quality Control in Health; URM: Fungal Library of the Federal University of Pernambuco.

Table 2
Half-maximal inhibitory concentration (IC_{50}) (μg/mL) fluconazole (FCZ) and essential oil leaves of *Piper regnellii* against *Candida* strains.

	CA INCQS 40006	CA URM 4127	CT INCQS 40042	CT URM 4262
EOLPR	419.94	263.82	802.95	804.39
FLUCONAZOLE	74.07	12.51	1378.5	135.45
EOLPR + FCZ	32.77	7.25	1410.5	36.14

CA: *Candida albicans*; CT: *Candida tropicalis;* FCZ: Fluconazole; EOLPR: Essential oil leaves of *Piper regnellii;* INCQS: National Institute for Quality Control in Health; URM: Fungal Library of the Federal University of Pernambuco.

On the other hand, the essential oil was found to inhibit the emission of filaments by *Candida strains* (Fig. 3). Accordingly, the emission of hyphae was observed only in the growth control group, since the treatment with the essential oil or fluconazole resulted in complete inhibition of hyphae growth, thus indicating a relevant inhibitory effect by these treatments on fungal morphological transition.

4. Discussion

Essential oils are composed of a variety of chemical substances derived from the secondary metabolism of vegetables (Del Quiqui, 2019). A review study on *Piper* species by (Salehi, 2019) Salehi et al. (2019) covering 130 species, including *P. regnellii*, found that only 16 of them presented detailed information regarding their chemical constitution.

The chemical analysis of essential oil obtained from fresh leaves of *P. regnellii* by Pessini et al. (2003) obtained a yield of 0.80%, corroborating the findings of the present study (yield = 0.85%). Terpenes and their derivatives stand out among the constituents of essential oils of several species, including *Piper* species, characterized by the abundance of monoterpenes, sesquiterpenes and phenylpropanoids (Bernuci et al., 2016). Phenylpropanoids are compounds chemically characterized by the presence of an aromatic ring attached to a chain of three carbons originated from the biosynthesis of shikimic acid (Simões et al., 2010).

With regard to the chemical profile, the data obtained in the present study differ from the results found by Pessini et al., (2003), who

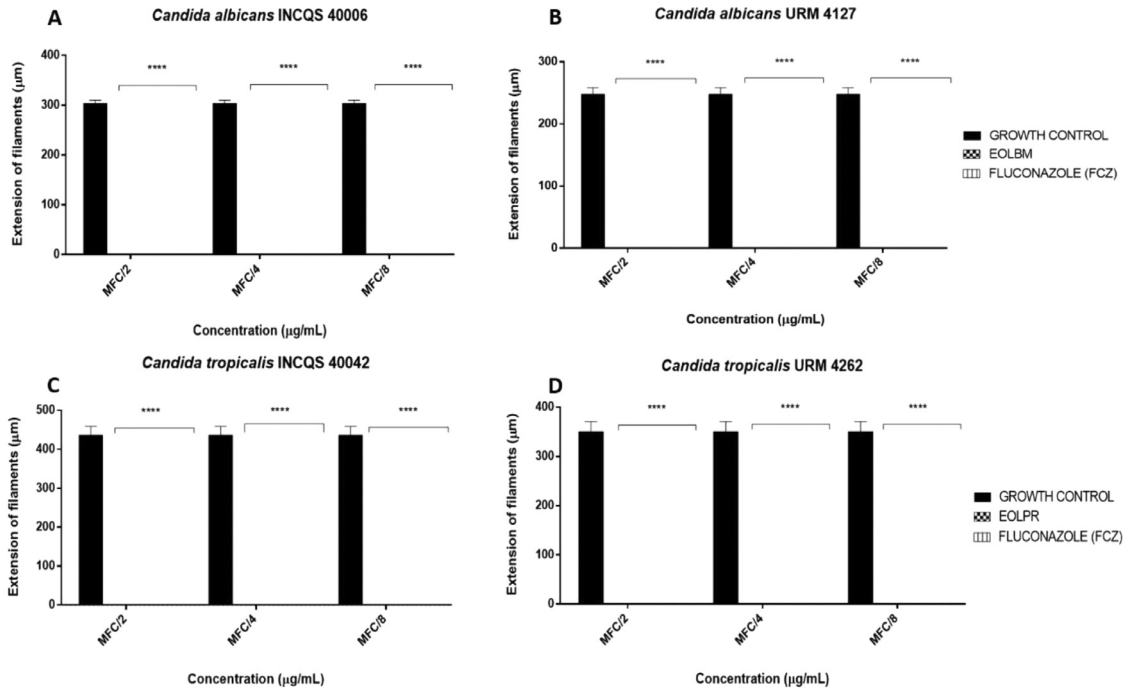

Fig. 3. Effects of fluconazole (FCZ) and EPLPR on fungal morphology. The emission of filaments by *C. albicans* strains CA INCQS 40006 (A) and CA URM 4227 and *C. tropicalis* (CT) strains CT INCQS 40042 (C) and CT URM 4262. INCQS: National Institute for Quality Control in Health; URM: Fungal Library of the Federal University of Pernambuco.

identified β-myrcene as the major component (70%) of the essential oil of *P. regnellii*, while our analysis found apiol as the predominant compound (70.79%). Constantin et al. (2001) evaluated the chemical composition of the essential oil of *Piper regnellii* leaves using samples collected in the state of São Paulo and demonstrated the presence of β-myrcene (52.6%), linalool (15.9%), β-caryophyllene (8.5%), and bicyclogermacrene (2.9%) as major compounds. It is noted that among these compounds, only bicyclogermacrene was identified in the present study.

Such differences reinforce the evidence suggesting that environmental factors such as the climate and soil characteristics, as well as collection and extraction conditions, in addition to genetic factors, influence the production of chemical compounds by a given species (Bernuci et al., 2016; Lucena, 2015). Accordingly, evidence has suggested that changes in seasonality can redirect metabolic pathways associated with the production of secondary metabolites (Del Quiqui, 2019; Salehi, 2019). In addition, a study on the variation of the volatile components of the essential oil of *Piper regnellii* leaves found differences in the chemical composition of samples collected at different times of the day, demonstrating the influence of the circadian cycle on the production of chemical compounds by plants (Anderson et al., 2018).

Evidence has shown that the antimicrobial activity of essential oils is intrinsically related to their hydrophobic characteristics, which favor their interaction with cell membrane lipids and bacterial mitochondria, increasing permeability and facilitating the leakage of molecules that play critical roles on cell functioning (Solórzano-Santos and Miranda Novales, 2012; Dhifi et al., 2016).

While the present study demonstrated no intrinsic antibacterial activity of the essential oil of *Piper regnellii*, a study by Constantin et al. (2001) demonstrated its antibacterial action against *S. aureus* and *Pseudomonas aeruginosa*. However, these authors used the disc diffusion methodology. In addition, the essential oil contained myrcene as the major compound (52.6%), which may explain the differences in the effects observed both studies, since studies have demonstrated that the composition of essential oils significantly contributes to their pharmacological effects (Falleh et al., 2020).

Studies have shown that *Piper* species, including *P. regnellii* (extracts), have a remarkable antibacterial action against strains of *S. aureus, P. aeruginosa*, and *Bacillus subtilis* (Pessini et al., 2003). In addition, natural products derived from *Piper* species have shown promising effects against Gram-positive and Gram-negative bacteria (Alves et al., 2016; Mgbeahuruike et al., 2017).

. However, this study demonstrated that the essential oil of *Piper regnellii* antagonized the activity of conventional antibiotics against *S. aureus* strains. Evidence indicates that Gram-positive bacteria are less susceptible to the action of essential oils since the hydrophobic character of these substances can impair their penetration through the cell wall (Lima, 2017). In addition, the presence of different constituents in essential oils can result in interactions that impair the antibacterial activity of synthetic or natural compounds (Falleh et al., 2020).

The essential oil *Piper regnellii* was found to potentiate the effects of gentamicin against *E. coli*, a Gram-negative bacterium. However, this effect was achieved at 20 μg/mL, which is 5-fold greater than the concentration defined as clinically relevant by the Clinical and Laboratory Standards Institute CLSI (2015). In fact, the presence of an outer layer of lipopolysaccharides on the cell wall of these bacteria seems to favor the toxic action of essential oil components (Dhifi et al., 2016), although the mechanisms by which substances derived from plant metabolism modulate antibiotic activity by resistant strains remain to be better understood (Sales, 2017; De Lima, 2020).

The essential oil of *Piper regnellii* demonstrated weak antifungal activity against *Candida albicans* and *Candida tropicalis*. Substances with strong antifungal activity are those with MIC values less than 500 μg / mL. (Sartoratto, 2004).

Accordingly, previous studies reported that other species of *Piper* also showed weak antifungal activity against *Candida albicans* and *Candida tropicalis* species (Bezerra et al., 2020; Carneiro et al., 2019). Studies with extracts of *Piper regnellii* demonstrate that this species has antifungal activity against *Candida albicans* (Pessini et al., 2003) . However, the biological properties of the genus *Piper* can vary significantly according to the chemical composition of the plant material (Bezerra et al., 2020).

The chemical compound apiol found as a major constituent in the essential oil of *Piper regnellii* demonstrated antifungal activity against *Aspergillus flavus, A. niger, A. fumigatus*, and *Cladosporium herbarum*. It has been suggested that this phenylpropanoid could inhibit ergosterol synthesis, affecting the permeability of the membrane and leading to the leakage of cellular components (Das et al., 2020).

According to Almeida et al. (2009), the antifungal activity of the essential oil of *Piper aduncum* against *Clinepellis perniciosa* can be attributed to the presence of high concentrations of apiol. On the other hand, despite the high concentrations of apiol and dilapiol, the antifungal activity demonstrated in the present study can be considered weak.

Despite the weak antifungal action, the essential oil of *Piper regnellii* potentiated the action of fluconazole against *Candida albicans* and *Candida tropicalis*, reducing the effective concentration of the drug. Fluconazole is the standard therapeutic option for most infections caused by *Candida* species. However, the emergence of resistant strains has increasingly limited the use of this drug (MC Alister and Shapiro, 2019). Thus, research involving the combined use of natural products with commercial drugs significantly contributes to the combat of antifungal resistance (Kioshima et al., 2019; Andrade-Neto et al., 2020; (Do Av, 2019).

Synergism resulting from the association of essential oils and commercial drugs has been associated with the complementarity of chemical components that constitute the complex mixture of substances found in essential oils (Bernuci et al., 2016; Anderson et al., 2018).

The high pathogenicity of *Candida species* is largely influenced by morphological transition mechanisms through which the cell changes from yeast to a filamentous structure, which also leads to antimicrobial resistance (Boni, 2017; MC Alister and Shapiro, 2019).

The essential oil of *Piper regnellii* completely inhibited the emission of hyphae by *Candida albicans* and *Candida tropicalis*, corroborating the results found by Carneiro et al. (2019) using essential oils of *Piper mikanianum* and *Piper diospyrifolium*, as well as the findings by Bezerra et al. (2020) using the essential oil of *Piper caldense*.

The emission of hyphae by fungi of the genus *Candida* significantly increases their invasive potential, contributing to a greater risk of generalized infections and increased mortality (MC Alister and Shapiro, 2019; Kornitz, 2019). The morphological transition involves a series of cellular and molecular events that lead to the activation of transcription factors that orchestrate the differential expression of a large variety of genes (Rocha et al., 2001; Cloutier et al., 2003; Kornitz, 2019). Therefore, it is hypothesized that the essential oil of *Piper regnellii* is interfering with the expression of genes and proteins that contribute to the morphological transition of *Candida species*. However, these mechanisms remain to be investigated at the molecular level.

Finally, it is noteworthy that the inhibition of the morphological transition by *Piper regnellii* can have a significant clinical impact since it interferes with one of the main virulence mechanisms in this genus.

5. Conclusion

The essential oil of *Piper regnellii* is chemically characterized by the abundance of phenylpropanoids, including apiol and dilapiol as major compounds. Regarding its intrinsic antimicrobial activity, this natural product presented weak antibacterial and antifungal activities. However, the association of the essential oil with gentamicin and fluconazole resulted in potentiating effects against *E. coli* and *C. tropicalis*, respectively.

The morphological transition of *C. albicans* and *C. tropicalis* was strongly inhibited by the treatment with the essential oil, highlighting the therapeutic potential of *P. regnellii*. However, further studies investigating the mechanisms underlying the modulation of this virulence factor are required for a better comprehension of the pharmacological properties of *P. regnellii* essential oil.

Declaration of Competing 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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