Original Article

STUDY OF THE ANTIFUNGAL POTENTIAL OF (*R*)-(+)-CITRONELLAL AND ITS ASSOCIATION WITH THERAPEUTIC AGENTS USED IN THE TREATMENT OF VULVOVAGINAL CANDIDIASIS

ESTUDO DO POTENCIAL ANTIFÚNGICO DO (R)-(+)-CITRONELAL E SUA ASSOCIAÇÃO COM AGENTES TERAPÊUTICOS UTILIZADOS NO TRATAMENTO DA CANDIDÍASE VULVOVAGINAL

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ABSTRACT: Vulvovaginal candidiasis (VVC) is a common fungal infection that affects healthy women of all ages. At least 75% of women will develop one or more infections once during their lifetime, with 6 to 9% of those individuals developing recurrent infections. In view of this context, this study sought to evaluate the antifungal potential of the isolated (*R*)-(+)-citronellal [(*R*)-(+)-CT] and associated to therapeutic agents of clinical importance. The enantiomer was solubilized in tween 80 and dimethylsulfoxide (DMSO). Posteriorly diluted in sterile distilled water up to the concentration of 2048µg/mL. The minimum inhibitory concentration (MIC) of the product was determined by microdilution in RPMI-1640 obtaining dilutions of 1024-4µg/mL. The minimum fungicidal concentration (MFC) was determined by the Sabouraud dextrose agar (SDA) depletion technique from aliquots of 1µL of the MIC, MIC × 2 and MIC × 4. The MIC and the MFC values of (*R*)-(+)-CT for 90% of the *C. albicans* strains were 16 and 32µg/mL respectively. In the susceptibility test, *C. albicans* presented a high resistance to fluconazole and to itraconazole, 12 (92.30%) of the strains. However, for ketoconazole and miconazole the resistance was of 4 (30.76%) and 3 (23.07%) of the strains respectively. In the combination testing of the (*R*)-(+)-CT with ketoconazole and miconazole, the resistance was completely reverted. For fluconazole and itraconazole, the resistance was reverted in 9 (75%) and 7 (58.33%) of the strains respectively. The (*R*)-(+)-CT presented fungicide activity with MFC of MIC × 2. When in combination with ketoconazole, fluconazole, itraconazole and miconazole increased the inhibition zones of these antifungal drugs, reducing the resistance against *C. albicans*.

KEYWORDS: Monoterpenoid, Anti-*C. albicans.* Antifungal agents. Secondary metabolites. Citronellal.

INTRODUCTION

Vulvovaginal candidiasis (VVC) affects 75% of all women at least once during their lifetime, occurring more frequently during fertile age (ADESIJI et al., 2011, GANDHI et al., 2015). Another smaller group of women (6-9%) experience the recurrence of this disease, called recurrent vulvovaginal candidiasis (RVVC) and defined as presenting at least 3 symptomatic episodes during the 12 previous months even though some researchers demand still, an additional episode (FOXMAN et al., 2013, JACK, SOBEL, 2016). Although various species of *Candida* have been involved in VVC and RVVC, *Candida albicans* is the predominant etiological agent, causing 85-95% of these infections (HONG et al., 2014, BEHZADI et al., 2015).

VVC can be manifested as a simple form, as sporadic cases of light infection caused by *C. albicans*. However, complex or complicated cases are caused by other species of the genus *Candida*, and severe infections, VVC during pregnancy and associated to other medical conditions, such as immunosuppression or diabetes (LI et al., 2014). However, it is difficult to assess the exact incidence of VVC, due to the high rate of indiscriminate use of medicines. Furthermore, the diagnosis is frequently and entirely based on signs and symptoms without any diagnostic tests to confirm, and the treatment depends on whether the infection is complex or simple (BEHZADI et al., 2015, DOVNIK et al., 2015).

A variety of antifungal agents have been widely used to treat these infections. The azoles including the ketoconazole, fluconazole, itraconozole and miconazole have been used in a variety of therapeutic schemes for the treatment of VVC and RVVC (SEKHAVAT et al., 2011). However, due to the dynamics of antimicrobial resistance, which involves a complex association of multiple factors inherent to both the host and to the

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fungus itself, therapeutic failure has been significant (ESPINEL-INGROFF, 2008). Particularly, the azoles, the most common antifungal medication used in this disease, have been presenting an unfavorable picture (PFALLER, 2012).

The essential oils, a large group of secondary metabolites of plants involved in the defense processes and synthesized in response to microbes and plague attacks by herbivore insects, display excellent antimicrobial, insecticide, as well as anticancer activity (PICHERSKY, GERSHENZON, 2002). The terpenoids are condensation products of isoprene, containing 5 carbon atoms in its structure. Are important components of essential oils with vast biological activity (SAMY, GOPALAKRISHNAKONE, 2008, LORENZI et al., 2009). The anti-*Candida* activity of essential oils extracted from plants has been reported in several scientific papers over the last years, and consequently the essential oils, as well as some of their phytoconstituents, are used topically in the form of creams, gels and pessaries for the treatment of microbial infections (MONDELLO et al., 2006).

Furthermore, there is a growing interest in the use of combination therapy in order to avoid the collateral effects associated with high doses or long-term usage of conventional drugs (WAGNER, 2006). It includes the use of combinations of synthetic substances, as well as natural products, together with conventional medicines against several infectious diseases, such as candidiasis. Some essential oils and phytoconstituents are reported to synergically improve the activity of antibiotics such as amphotericin B, ketoconazole and fluconazole (ROSATO et al., 2008, HEMAISWARYA et al., 2008, WAGNER, ULRICH-MERZENICH, 2009).

The phytoconstituent (*R*)-(+)-CT, belonging to the group of the monoterpenoids, is one of the major substances of essential oils of aromatic plants, such as the ones of the genus *Cymbopogon* and *Eucalyptus* (AVOSEH et al., 2015, BATUBARA et al., 2015). The citronellal is isolated as a non-racemic mixture of the *R* and *S* enantiomers, and has shown to have several biological activities, among them, antimicrobial, antioxidant, herbicide, insecticide and repellent action (SCHERER et al., 2009, QUINTANS-JÚNIOR et al., 2011, BRITO et al., 2012, TOMAZ et al., 2014). In this context, it was aimed to assess the antifungal potential of the isolated (*R*)-(+)-CT, and associated to therapeutic agents of clinical importance frequently used in the treatment of the vulvovaginal candidiasis.

MATERIAL AND METHODS

Phytoconstituent, antifungal standards and substances

The following substances used in this work were obtained commercially: enantiomer (*R*)-(+)-CT [(3*R*)-3.7-dimethyloct-6-enal] (Figure 1), (Purity > 90%), dimethylsulfoxide (DMSO) and tween 80 (0.02%) (all from Sigma-Aldrich, São Paulo, SP, Brazil). The tween 80 and the DMSO were solubilized in a proportion that did not exceed 0.5% in the test, and was posteriorly diluted in sterile distilled water in order to reach the initial concentration of 2048µg/mL (HOOD et al., 2003, BRUNI et al., 2004; NASCIMENTO et al., 2007; PEREIRA et al., 2014). Furthermore, ketoconazole, fluconazole, itraconazole and miconazole were respectively, purchased from Control Center and Products for Diagnosis (CECON) Ltd. (São Paulo, SP, Brazil).

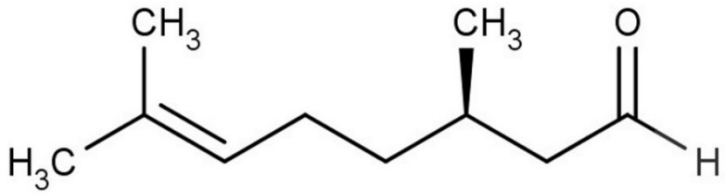

Figure 1. Struture of Citronellal ((*R*)-3,7- dimethyloct-6-enal)

Culture media

To test the biological activity of the products, Sabouraud dextrose broth (SDB) and Sabouraud dextrose agar (SDA) were purchased from Difco Laboratories (Detroit, MI, USA). Furthermore, RPMI-1640-L-glutamine (without sodium bicarbonate) (Sigma-Aldrich, São Paulo, SP, Brazil) culture media were used. They were prepared and used according to the manufacturers' instructions.

Fungal strains

The assays were performed with 13 strains of *C. albicans*: LM 852, LM 157, LM 152, LM 240,

LM 0202, LM 246, LM 228, LM 227, LM 319, LM 16, and LM 15 (isolated from vaginal), and two standard *C. albicans* strains: ATCC 76485 and ATCC 76645. All strains belong to the collection of the Mycology Laboratory, Department of Pharmaceutical Sciences, Federal University of Paraíba (LM, DCF, UFPB). These strains were maintained in SDA at 35±2 °C and 4 °C until used in tests.

Inoculum

The suspensions were prepared from recent *C. albicans* cultures, plated on SDA, and incubated at 35±2°C for 24-48h. After incubation, was transferred roughly 4-5 yeast colonies (with a sterile loop) to test tubes containing 5.0mL of sterile saline (NaCl 0.85%). The resulting suspensions were stirred for 15 seconds with the aid of a Vortex apparatus (Fanem Ltd., Guarulhos, SP, Brazil). The turbidity of the final inoculum was standardized using a barium sulfate suspension (tube 0.5 on the McFarland scale). The final concentration obtained was about 1-5 × 10^5 colony forming units per milliliter (CFU/mL) (KONEMAN et al., 2008; OSTROSKY et al., 2008).

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The determination of the products' MIC on the thirteen strains used in the biological assays was determined by the broth microdilution method (CLEELAND, SQUIRES, 1991, HADACEK, GREGER, 2000, NCCLS/CLSI, 2002). One hundred microliters (100µL) of liquid medium RPMI-1640 was transferred into the wells of a 96-well microdilution plate with a "U" shaped bottom (Alamar, Diadema, SP, Brazil). Then, 100µL of (*R*)-(+)-CT emulsion was inoculated in the first horizontal row of the plate wells. Doubled serial dilutions, where a 100µL aliquot removed from the most concentrated well went to the next well, yielded concentrations of 1024-4µg/mL. Finally, 10µL of *C. albicans* inoculum suspension was added to each well of the plate, where each column represented a yeast strain. In parallel, controls were made for yeast viability and for susceptibility with the standard antifungal nystatin (100IU/mL). The plates were incubated at 35±2°C for 24-48h. After the appropriate incubation time, the presence (or absence) of growth was observed visually. The formation of cell clusters or "buttons" in the plate wells was considered. The MIC was defined as the lowest (*R*)-(+)-CT concentration that produced visible inhibition of yeast growth.

The antimicrobial activity of the products was interpreted (considered active or not), according to the criteria proposed by Morales et al., 2008: strong/good activity (MIC: <100µg/mL); moderate activity (MIC: 100-500µg/mL); weak activity (MIC: 500-1000µg/mL); and inactive product/no antimicrobial effect (MIC: >1000µg/mL).

To determine the MFC, we subcultured 1 µL aliquots of MIC, MIC × 2 and MIC × 4 of the product, nystatin (100IU/mL), and the control yeast growth onto Petri dishes containing SDA. After 24-48 hours of incubation at 35±2°C, a reading was made to evaluate the MFC, as based on the growth of the controls. The MFC was defined as the lowest product concentration that inhibited growth of the yeast or permitted less than three CFUs to occur, resulting thus in 99.9% fungicidal activity (ERNST et al., 1996, ESPINEL-INGROFF, 2002).

Biological activity assays were performed in duplicate, and the results were expressed as the arithmetic mean of the MIC and MFC.

Susceptibility assays

The fungal susceptibility test was carried out based on the disk-diffusion method in solid mean (BAUER, et al., 1966; KONEMAN et al., 1993; HADACEK, GREGER, 2000). In this test, the following antifungal medications were used: ketoconazole (50µg), fluconazole (25µg), itraconozole (10µg) and miconazole (50µg). The interpretation of the results was carried out using the sensitive or resistant criteria recommended by the (CECON) Ltd. (São Paulo, SP, Brazil) and the CLSI, 2009.

Combination studies in vitro

The susceptibility tests of the combination of (*R*)-(+)-CT with the antifungal agents were also carried out based on the disk-diffusion method in solid media (OLIVEIRA et al., 2006, OSTROSKY et al., 2008).

In this test, the antifungal disks in their respective concentrations were soaked with 10µL of the MIC of (*R*)-(+)-CT, and posteriorly dispensed in Petri dishes containing SDA inoculated with 1mL of the fungal suspensions. Then, the dishes were incubated at 35±2°C for 24-48h. The interactions of the (*R*)-(+)-CT with the antifungal agents were considered as being positive (synergism), when the inhibition zone of the combined application was (≥ 2mm) in relation to the antifungal medication alone, and as being negative (antagonism), when the inhibition zone of the association was (≤ 2mm) to the presented by the isolated antifungal medication and " 0 interaction" (indifferent), when the inhibition

zone of the combination was the same as the antifungal medication alone (CUENCA-ESTRELLA, 2004, CLEELAND, SQUIRES, 1991). The tests were carried out in duplicate and the results were expressed by the arithmetic mean of the diameters formed in the two tests in parallel.

Statistical analysis

For the statistical treatment the GraphPad Prism 6.0 software was used, and for the analysis between the columns the Student's t test was applied, and the results were considered significant when $p \leq 0.05$.

RESULTS

The results of the antifungal activity of the (*R*)-(+)-CT against the *C. albicans* strains were determined using the MIC and MFC by micro-dilution in broth. The MIC values of the (*R*)-(+)-CT varied between 32 and 16µg/mL, however the latter corresponds to the inhibition of fungal growth in 90% of the tested strains (Table 1).

Table 1. MIC_{90} values (µg/mL) of (*R*)-(+)-CT against *C. albicans* strains by broth microdilution.

Fungal strains / Treatment	LM 852	LM 157	LM 152	LM 240	LM 0202	LM 246	LM 228	LM 227	LM 319	LM 16	LM 15	ATCC 76485	ATCC 76645
1024µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
512µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
256µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
128µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
64µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
32µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
16µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	-
Negative control	-	-	-	-	-	-	-	-	-	-	-	-	-
Positive control	+	+	+	+	+	+	+	+	+	+	+	+	+

(+) inhibition (-) no inhibition

The MFC was of 32µg/mL, corresponding to the MIC × 2 for 90% *C. albicans* population as can be observed in (Table 2).

The results of the fungal susceptibility tests for *C. albicans* for the standard antifungal agents were determined by the disk-diffusion test in solid mean. The resistance profile was observed for 12 (92.30%) of the fungal strains to the fluconazole and to itraconazole. However, for ketoconazole and miconazole the resistance was of 4 (30.76%) and 3 (23.07%) of the strains respectively (Table 3).

Table 2. MFC_{90} values (µg/mL) of (*R*)-(+)-CT against *C. albicans* strains.

Fungal strains / Treatment	LM 852	LM 157	LM 152	LM 240	LM 0202	LM 246	LM 228	LM 227	LM 319	LM 16	LM 15	ATCC 76485	ATCC 76645
1024µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
512µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
256µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
128µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
64µg/mL	+	+	+	+	+	+	+	+	+	+	+	+	+
32µg/mL	+	+	+	+	+	+	+	+	+	+	-	+	+
Negative control	-	-	-	-	-	-	-	-	-	-	-	-	-
Positive control	+	+	+	+	+	+	+	+	+	+	+	+	+

(+) inhibition (-) no inhibition

Table 3. Susceptibility testing of *C. albicans* strains to standard antifungal. Average diameters of halos expressed in (mm).

Fungal strains / Treatment	LM 852	LM 157	LM 152	LM 240	LM 0202	LM 246	LM 228	LM 227	LM 319	LM 16	LM 15	ATCC 76485	ATCC 76645	Classification
KET (50µg)	14^{**}	0^{**}	22^{*}	25^{*}	22^{*}	25^{*}	24^{*}	22^{*}	20^{**}	12^{**}	22^{*}	26^{*}	26^{*}	>20 (S) ≤20 (R)
FLU (25µg)	0^{**}	0^{**}	12^{**}	0^{**}	0^{**}	17^{**}	20^{*}	0^{**}	16^{**}	0^{**}	0^{**}	0^{**}	0^{**}	≥20 (S) <20 (R)
ICZ (10µg)	0^{**}	0^{**}	0^{**}	15^{**}	0^{**}	18^{**}	20^{*}	12^{**}	18^{**}	12^{**}	0^{**}	16^{**}	13^{**}	≥20 (S) <20 (R)
MCZ (50µg)	23^{*}	22^{*}	27^{*}	23^{*}	20^{**}	30^{*}	30^{*}	22^{*}	20^{**}	24^{*}	25^{*}	20^{**}	28^{*}	>20 (S) ≤20 (R)
Control yeast	+	+	+	+	+	+	+	+	+	+	+	+	+	--

*Sensible (S); **Resistant (R), KET (Ketoconazole); FLU (Fluconazole); ICZ (Itraconazole); MCZ (Miconazole)

The results for the combination tests are shown in the (Table 4), where can be observed that the effects of the (*R*)-(+)-CT interference on the antifungal medications varied according to the type

of the therapeutic agent and the fungal strain tested. However, synergism was predominant on the four tested antifungal medications. The association of the (*R*)-(+)-CT with ketoconazole, as well as to miconazole, resulted respectively in synergetic effect in 13 (100%) of the fungal strains. The (*R*)-(+)-CT in combination with fluconazole showed synergism in 11 (84.61%) of the yeast, and in association with itraconazole 9 (69.23%).

Table 4. Average diameters (in mm) of the test (*R*)-(+)-CT combination of patterns and antifungal against *C. albicans* in solid medium.

Fungal strains / Treatment	**LM 852**	**LM 157**	**LM 152**	**LM 240**	**LM 0202**	**LM 246**	**LM 228**	**LM 227**	**LM 319**	**LM 16**	**LM 15**	**ATCC 76485**	**ATCC 76645**
KET (50µg)	25↑	50↑	35↑	45↑	40↑	45↑	42↑	35↑	40↑	34↑	40↑	50↑	45↑
FLU (25µg)	20↑	25↑	20↑	25↑	12↑	20↑	25↑	0I	14↓	20↑	20↑	30↑	30↑
ICZ (10µg)	20↑	0I	25↑	15I	20↑	24↑	20I	15↑	20↑	25↑	17↑	25↑	0↓
MCZ (50µg)	40↑	35↑	42↑	35↑	42↑	40↑	35↑	35↑	45↑	40↑	35↑	40↑	35↑
Control yeast	+	+	+	+	+	+	+	+	+	+	+	+	+

↑Synergism; ↓ Antagonism; I Indifferent

Furthermore, it was also observed that for some of the strains previously resistant to isolated antifungal medications, became sensitive when faced with the combination of the phytoconstituent with the antifungal agents.

For ketoconazole, the strains that suffered exchange of profile from resistant to sensitive were *C. albicans* LM 852, LM 157, LM 319 and LM 16 (Table 3, 4 and Figure 2).

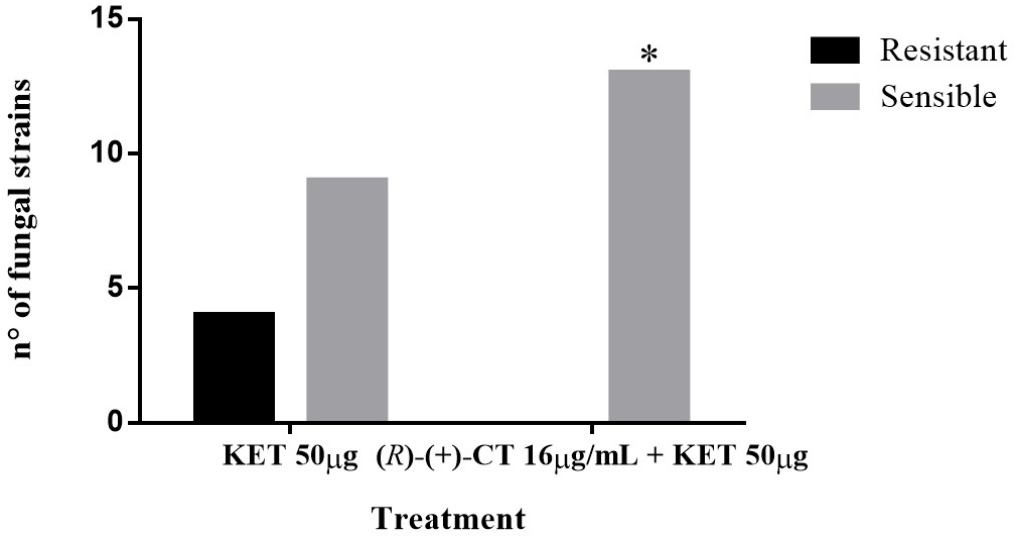

Figure 2. Resistance profile and sensitivity of *C. albicans* front ketoconazole and in combination with (*R*)-(+)-CT, $*p \leq 0.05$.

For the fluconazole, the combination with (*R*)-(+)-CT resulted in the change of resistance profile from resistant to sensitive of the following strains of *C. albicans*: LM 852, LM 157, LM 152, LM 240, LM 246, LM 16, LM 15, ATCC 76485 and ATCC 76645 (Table 3, 4 and Figure 3).

For the itraconazole, the effect of the combination with the phytoconstituent resulted in the change of the resistance profiles of the following yeasts: LM 852, LM 152, LM 0202, LM 246, LM 319, LM 16 and ATCC 76485 (Table 3, 4 and Figure 4).

As for the miconazole, there was a change of the resistance profile in three strains as a consequence of the combination of the (*R*)-(+)-CT with the antifungal agent: LM 0202, LM 319 and ATCC 76485 (Table 3, 4 and Figure 5).

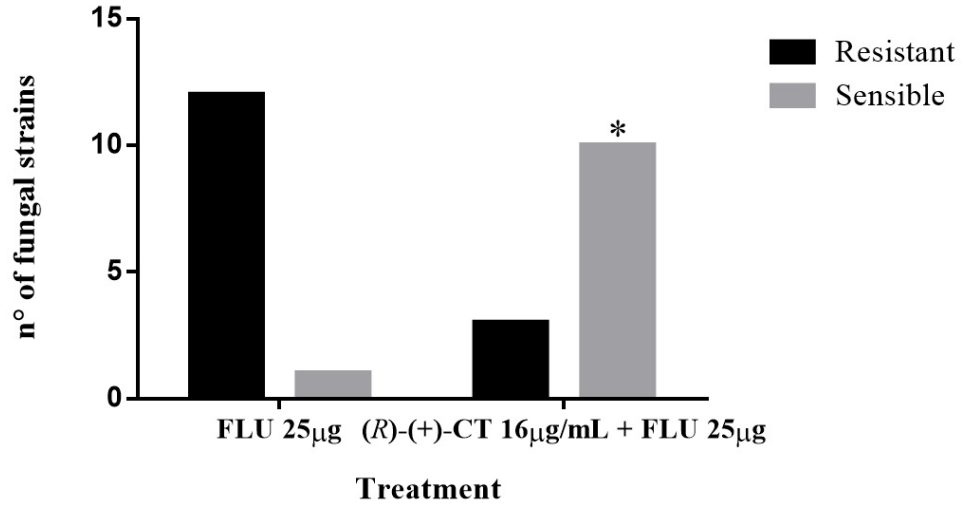

Figure 3. Resistance profile and sensitivity of *C. albicans* front fluconazole and in combination with (*R*)-(+)-CT, $*p \leq 0.05$.

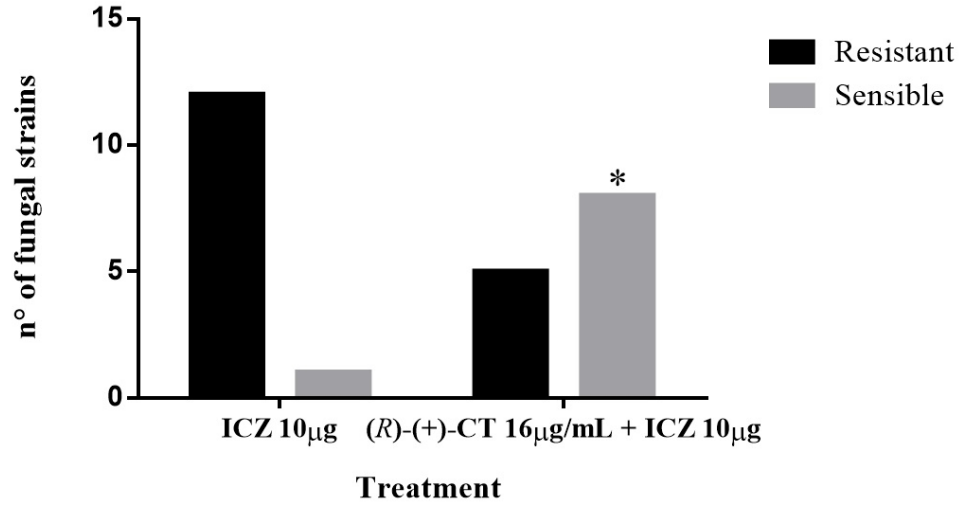

Figure 4. Resistance profile and sensitivity of *C. albicans* front itraconazole and in combination with (*R*)-(+)-CT, $*p \leq 0.05$.

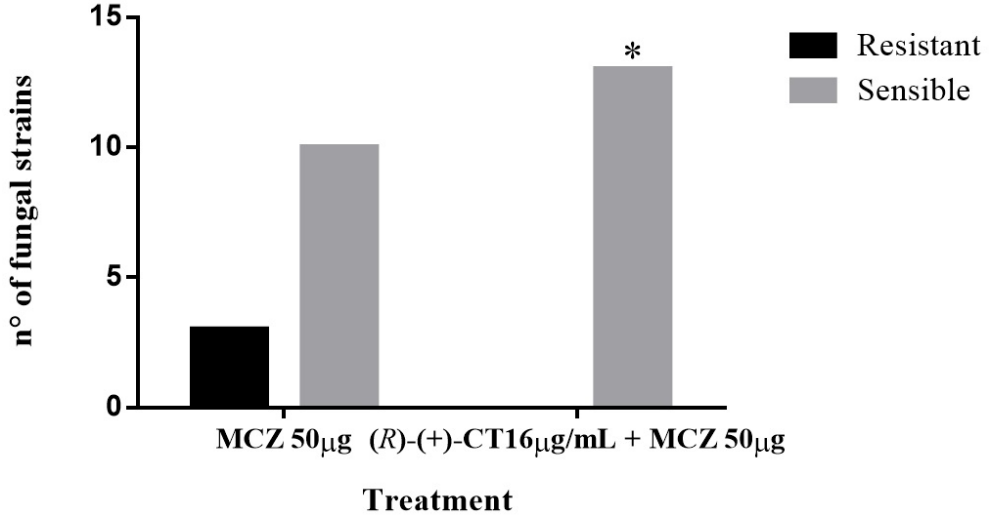

Figure 5. Resistance profile and sensitivity of *C. albicans* front miconazole and in combination with (*R*)-(+)-CT, $*p \leq 0.05$.

DISCUSSION

The high incidence of infections by *C. albicans* and the emergence of resistance to the antifungal drugs accentuates the need of studying new sources of drugs, such as natural products and their phytoconstituents (LIMA et al., 2012).

Herbal substances are used in the treatment of several diseases, but their application as a potential source of new drugs is still very little exploited. Of the 250.000 to 500.000 plant species, only a small percentage have had, their pharmacological properties studied (RATES, 2001). Thus, the compounds derived from plants are potential sources of new and valuable therapeutic agents (TROMBETTA et al., 2005, SINGH et al., 2011). Therefore, the phytoconstituents are important, due to the various pharmacological activities such as the antifungal, antibacterial and antiparasitic (OLAGNIER et al., 2007, CARDOSO et al., 2010, ZORE et al., 2011a).

The terpenoids such as the (*R*)-(+)-CT, a major phytoconstituent of the essential oils of plants of the genus *Cymbopogon* and *Eucalyptus* presents excellent antifungal activity (AVOSEH et al., 2015, BATUBARA et al., 2015). In this study, it was observed that the (*R*)-(+)-CT showed excellent antifungal efficiency in 90% of the *C. albicans* strains. According to the criteria of Morales et al., 2008, this phytoconstituent showed strong anti-*C. albicans* activity because the value of the MIC_{90} was less than 100µg/mL (MIC <100µg/mL). In literature, (*R*)-(+)-CT, also showed to have a good fungicide, bactericidal, trypanocidal and leishmanicidal activity (ZORE et al., 2011b, PEREIRA et al., 2015).

In this work, the fungicide effect of the (*R*)-(+)-CT was also verified in 90% of the *C. albicans* strains (MFC_{90} = 32 µg/mL). According to Hafidh et al., 2011 the fungicide effect of a natural product such as the citronellal, is observed when the coefficient between the MFC/MIC is between 1 and 2.

The treatment recommendations for VVC are separated into simple treatment, caused by *C. albicans* and complicated VVC, which includes RVVC, and severe VVC caused by non-*albicans* species, and VVC in immunocompromised hosts (WORKOWSKI, BERMAN, 2006, JACK, SOBEL, 2016).

The good performance evidenced for the ketoconazole and the miconazole, against *C. albicans* has already been observed, and the oral administration of ketoconazole has proved to be efficient against RVVC when faced with strains susceptible to the azoles in the maintenance therapy (SOBEL, 1985). However, due to the hepatic toxicity mainly to the ketoconazole, other therapeutic schemes are now preferred (LEWIS et al., 1984).

In this context, it has been observed that for the ketoconazole and the miconazole, *C. albicans* presents a good sensitivity, as shown in (Table 3). However, the presence of 30.76 and 23.07% of resistance to the *C. albicans* strains in this study emphasizes the change to a lower susceptibility to these antifungal medications (DALAZEN et al., 2011). Therefore, the high frequency of resistance to the triazolic drugs worked in this study, demonstrates a growing profile of resistance to the genus *Candida* (RUIZ-CAMPS, CUENCA-ESTRELLA, 2009).

For more than one decade, cases of reduced sensitivity to the fluconazole and itraconazole have been observed (MÍMICA et al., 2009, FAVALESSA et al., 2010), with the observation of cross-resistance to isolated *C. albicans* and non-*albicans*, by the previous and prolonged exposure to the fluconazole (NUNES et al., 2011). However, a lower susceptibility to the fluconazole and itraconazole has been observed, reported in vulvovaginal clinical samples (Table 3) (DALAZEN et al., 2011, ABACI, HALIKI-UZTAN, 2011). This way, *C. albicans* has shown to be predominantly resistant resembling the profile of this work (SPAMPINATO, LEONARDI, 2013).

In view of this context, the reality of the current clinical situation of the emerging cases of antimicrobial resistance, the treatment of infections by *C. albicans* and several other microorganisms has become more difficult, reflecting in a higher frequency of therapeutic failure to the monotherapy (AHMAD et al., 2010).

In these cases, the research of the interactions of natural and synthetic products on the effectiveness of the conventional antifungal medications seems to us as being quite promising if the combination results in a better activity spectrum and reduced toxicity in comparison to complementary schemes of a single agent (ROLING et al., 2002, AHMAD et al., 2010). This way, it seems that the modification of the antimicrobial activity resulting from the associations, with the expansion of the sensitivity profile of resistant fungal strains shows to be a new strategy in clinical practice, with the potential of being a modifier of the resistance profile (CUENCA-ESTRELLA, 2004, OLIVEIRA et al., 2006, SOUSA et al., 2011, RIBEIRO et al., 2013).

The terpenoids' mechanisms of anti-*Candida* activity are not very clear, but have been reported to modulate the mevalonate pathway (MP), alter the cellular levels of intermediate molecules and functions associated in eukaryotic cells (BREHM-STECHER, JOHNSON, 2003). In addition to the modulation of the MP, terpenoids have been reported to destabilise the membrane and modular functions associated to the membrane, such as permeability, cell signaling, leading to cell death (TROMBETTA et al., 2005; ZORE et al., 2011a), (BREHM-STECHER, JOHNSON, 2003; MO, ELSON, 2004; GOULART et al., 2004).

The terpenoids have also been reported to detain the cell cycle in eukaryotic cells, and the citronellal detains the cell cycle of *C. albicans* in the DNA duplication phase, called S phase (Zore et al., 2011a; BREHM-STECHER, JOHNSON, 2003; MO, ELSON, 2004; GOULART et al., 2004).

It is probable that due to the level of lipophilicity, the (*R*)-(+)-CT has interacted with the components of the fungal membrane´s phospholipid bilayer therefore affecting , the degree of fluidity , besides interfering in signaling routes involved in the synthesis of polysaccharides such as the β-glucan, mannan and chitin, important for the maintenance of the *C. albicans* cell wall. Therefore, these interactions may cause a greater influx of antifungal agents, resulting in the increase of the inhibition zones and thus reducing the resistances of these yeasts (Table 4) (SANCHEZET et al., 2004; BRAGA et al., 2007; ZORE et al., 2011b).

CONCLUSION

Citronellal has a promising fungicide activity, being capable of inhibiting an infection still in its initial stage. In addition, this monoterpene (*R*)-(+)-CT has shown to act synergically with ketoconazole, miconazole, fluconazole and itraconazole. Thus, this tested compound may become an alternative in the monotherapeutic antifungal chemotherapy for VVC and RVVC or in combination with conventional drugs. However, there is a need for more studies aimed to correlate its potent antifungal activity *in vitro* and *in vivo* proving its security for clinical application.

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RESUMO: Candidíase vulvovaginal (CVV) é uma infecção fúngica comum que afeta mulheres saudáveis de todas as idades. Pelo menos 75% das mulheres irão desenvolver uma ou mais infecções uma vez durante a vida, com 6 a 9% dos indivíduos desenvolvendo infecções recorrentes. Diante deste contexto, buscou-se avaliar neste estudo o potencial antifúngico do (*R*)-(+)-citronelal [(*R*)-(+)-CT] isolado e associado a agentes terapêuticos de importância clínica. O enantiômero foi solubilizado em tween 80 e dimetilsulfóxido (DMSO). Posteriormente diluiu-se em água destilada estéril até a concentração de 2048µg/mL. A concentração inibitória mínima (CIM) do produto foi determinada por microdiluição em meio RPMI-1640 obtendo diluições de 4-1024µg/mL. A concentração fungicida mínima (CFM) foi determinada pela técnica de esgotamento em agar Sabouraud dextrose (ASD) a partir de alíquotas de 1mL da CIM, CIM × 2 e CIM × 4. A CIM e a CFM do (*R*)-(+)-CT para 90% das cepas de *C. albicans* foram 16 e 32µg/mL respectivamente. No ensaio de suscetibilidade, *C. albicans* apresentou alta resistência ao fluconazol e ao itraconazol, 12 (92.30%) das cepas. No em tanto, para o cetoconazol e o miconazol a resistência foi de 4 (30.76%) e 3 (23.07%) das cepas respectivamente. No ensaio de combinação do (*R*)-(+)-CT com cetoconazol e miconazol, a resistência foi completamente revertida. Para o fluconazol e o itraconazol, a resistências foi revertida em 9 (75%) e 7 (58.33%) das cepas respectivamente. O (*R*)-(+)-CT apresentou atividade fungicida com CFM igual à CIM × 2. Quando em combinação com cetoconazol, fluconazol, itraconazol e miconazol ampliou as zonas de inibição desses antifúngicos, diminuindo a resistência contra *C. albicans*.

PALAVRA-CHAVE: Monoterpenoide. Anti-*C. albicans*. Agentes antifúngicos. Metabólitos secundários. Citronelal

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