Article

In vitro antifungal activity and cytotoxic effect of essential oils and extracts of medicinal and aromatic plants against *Candida krusei* and *Aspergillus fumigatus*

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RESUMO: "**Atividade antifúngica *in vitro* e os efeitos citotóxicos de óleos essenciais e extratos de plantas medicinais e aromáticas contra *Candida krusei* e *Aspergillus fumigatus***" As plantas são geralmente utilizadas na medicina tradicional como agentes antimicrobianos e seus óleos essenciais e extratos foram conhecidos por possuir atividade antifúngica. O objetivo deste estudo foi avaliar *in vitro* a atividade de 32 óleos essenciais e 29 extratos contra *Candida krusei* e *Aspergillus fumigatus*, bem como o efeito citotóxico em células Vero. A curva do tempo-morte e a interação entre antifúngicos e *Chenopodium ambrosioidese* do extrato de *Myrcia cucullata* mostraram atividade antifúngica contra *C. krusei* (geometric means of the minimal inhibitory concentration [GM-MIC] 7,82 e 31,25 μg/mL, respectivamente). *Lippia citriodora* foi ativa contra *C. krusei* e *A. fumigatus* (GM-CIM = 99,21 μg/mL e 62,5 μg/mL, respectivamente). Os testes de tempo-morte feitos com óleo de *C. ambrosioides* mostraram atividade fungicida em 4x MIC. A interação do óleo *C. ambrosioides* com itraconazol e anfotericina B foi testada pela técnica de xadrez. Nenhuma interação foi detectada pela combinação do óleo *C. ambrosioides* com anfotericina B e itraconazol (intervalo fractional inhibitory index [FICI] = 1,03-1,06 e 1,03-1,00, respectivamente). Os ensaios de citotoxicidade para todas as amostras foram realizadas com MTT. Apenas os óleos *Hedyosmun* sp. e *L. dulcis* foram citotóxicos.

Unitermos: *Candida krusei, Aspergillus fumigatus,* óleos essenciais, extratos, curva do tempo-morte, técnica de xadrez.

ABSTRACT: The plants are usually used in traditional medicine as antimicrobial agents and their essential oils and extracts have been known to possess antifungal activity. The aim of this study was to evaluate the activity of 32 essential oils and 29 extracts *in vitro* against *C. krusei* and *A. fumigatus* as well as the cytotoxic effect on Vero cells. Time-kill curve and interaction between antifungal and the most active sample against *C. krusei,* was also evaluated. The oils from *C. ambrosioides* and the extract of *M. cucullata* showed antifungal activity against *C. krusei* (GM-MIC 7.82 and 31.25 μg/mL, respectively). *L. citriodora* was active against *C. krusei* and *A. fumigates* (GM-MIC = 99.21 μg/mL and 62.5 μg/mL respectively). Time-kill assays done with *C. ambrosioides* oil showed fungicidal activity at 4x MIC. The interaction of *C. ambrosioides* oil with itraconazole and amphotericin B was tested following the chequerboard technique. No interaction was detected for the combination of *C. ambrosioides* oil with amphotericin B and itraconazole (FICI range = 1.03-1.06 and 1.03-1.00, respectively). Cytotoxicity assays for all samples were carried out with MTT. Only the oil from *Hedyosmun* sp. and *L. dulcis* were cytotoxic.

Keywords: *Candida krusei, Aspergillus fumigatus,* essential oil, extract, checkerboard, time-kill curve.

INTRODUCTION

Candida spp. and *Aspergillus* spp. are responsible for the majority (80 to 90%) of fungal infections (Espinel-Ingroff et al., 2005). *Candida* species produce infections ranging from non-life-threatening mucocutaneous illnesses to invasive processes that may involve any organ. *C. albicans* is the predominant species causing infection; however infections due to other species such as *C. krusei*, associated with intrinsic resistance to fluconazole, decreased susceptibility to flucytosine and amphotericin B, and resistance to echinocandins, are on the increase (Capoor et al., 2005, Cantón et al., 2008, Pfaller et al., 2008). Additionally, *C. krusei* has the ability to adhere to a host surface or a prosthesis leading to the formation of biofilms which further facilitate adhesion and infection (Parahitiyawa et al., 2006).

Aspergillosis encompasses a broad spectrum of diseases caused by members of the genus *Aspergillus,* the most frequently responsible species are *A. flavus* and *A. fumigatus.* The last specie is one of the major causes of contamination of intrahospital environments, infecting patients by inhalation, direct inoculation through surgical tools, mechanical ventilation, air conditioners or filters (Haiduven, 2008). Additionally, the emergence of resistance of this fungus to itraconazole and elevated minimum inhibitory concentrations (MIC) to voriconazole, posaconazole, and the experimental azole, ravuconazole has appeared. A. fumigatus has also displayed variable susceptibility to amphotericin B (Snelders et al., 2008, Qiao et al., 2008).

The development of fungal drug resistance, drug-related toxicity, significant drug interactions and insufficient bioavailability of the conventional antifungal drugs, has encouraged the search for new alternatives among natural products (Cavaleiro et al., 2006). Plants are usually used in traditional medicine as antimicrobial agents and their essential oils and extracts have been known to possess antibacterial and antifungal proprieties (Cowan, 1999; Tempone et al., 2008). Previous reports have suggested that several essential oils and extracts show important antifungal activity against yeasts, dermatophytes and *Aspergillus* isolates, and have therapeutic potential, mainly against fungal diseases involving mucosal, cutaneous and respiratory tract infections (Cavaleiro et al., 2006; Tempone et al., 2008; Tavares et al., 2008).

The aim of this study was to evaluate the activity against *C. krusei* and *A. fumigatus, in vitro*, as well as the cytotoxic effect of essential oils and extracts of Colombian plants. Additionally, the combined effects of itraconazole and amphotericin B, and the pharmacodynamics with the most active samples were evaluated against *C. krusei* by the chequerboard method and the time-kill curves, respectively

MATERIALS AND METHODS

Plant material

Stems, leaves, flowers and roots of 57 medicinal and aromatic plants were collected in different regions of Colombia as part of a survey conducted by CENIVAM, a Research Centre devoted the study of aromatic plants and medicinal in Colombia. The taxonomic identification of the botanical samples was performed by Dr. Jose Luis Fernandez at the National Herbarium from Colombia, Institute of Natural Sciences, Faculty of Sciences, Universidad Nacional de Colombia, Bogota, where voucher specimens were deposited.

The plants selected belong to the following genera: *Salvia* (*S. aratocensis* subsp. *suratensis* 516326, *S. rubriflora* Kunth 521064, *S. amethystina* J.E. Smith 521071, *S. bogotensis* Benth 521063, *S. rubriflora* Epling 521107, *S. melaleuca* subsp. *totensis* 521038, *S. melaleuca* subsp. *melaleuca* 521076, *S. bogotensis* 521074), *Lippia* (*L. citriodora* 484334, *L. dulcis* 512079, *L. origanoides* 512075, *L. citriodora* 480749, *L. schlimii* Turcz 521078), *Lepechinia* (*L. salviifolia* Kunth subsp. *salviifolia* 521070, *L. conferta* 521068), *Hyptis* (*H. suaveolens* (L) Poit 512267, *H. perbullata* 521095), *Ocimum* (*O. campechianum* 512263, *O. tenuiflorum* L. 516925), *Croton* (*Croton* spp. 519596, *C. leptostachyus* 519598), *Lantana* (*L. camara* 520293, *L. fucata* Lindl. 521031). Additionally, the following plants were also selected, *Swinglea glutinosa* 521530, *Pimienta racemosa* 512227, *Siparum conica* 519971, *Nectandra acutifolia* 520339, *Calycolpus moritzianus* 517006, *Artemisia vulgaris* L. 517002, *Turnera* aff. diffusa Willd. ex Schult. 516293, *Rosmarinus officinalis* 531011, *Hedyosmum scaberrium* Standl. 517321, *Satureja brownei* (Sw.) Briqn 519796, *Aloysia tripilla* 517189, *Chenopodium ambrosioides* L. 519592, *Morella parvifolia* (Benth) 517726, *Schinus molle* L. 517747, Calyptranthes sp. 517736, *Bursera graveolens* (Kunth) Triana & Planch. 517753, *Cymbopogon citratus* 531013, *Aristolochia anguicida* 512207, *Morinda royoc* L. 512222, *Eugenia* cf. *uniflora* 512226, *Stachytarpheta mutabilis* (Jacq) Vahl 512281, *Calea glomerata* 512231, *Myrcia cucullata* 512228, *Tagetes lucida* 512074, *Hedyosmum racemosum* 517005, *Psychotria poeppigiana* müll Arg. 512218, *Phyllanthus acuminatus* 512239, *Eriope crassipes* Benth 512260, *Guatteria metensis* 512205, *Aristolochia ringens* 512238, *Coccocypselum hirsutum* Bartl 519995, *Alternanthera lanceolata* 519981, *Baccharis prunifolia* 521077, and *Sigesbeckia agrestis* 521062.

Extracts and essential oils extraction

Essential oils (32 oils) and extracts (29) were evaluated. The essential oils were extracted from dried stems, leaves, flowers and roots (300 g) by microwave-assisted hydrodistillation (30 min, 250 mL water), using

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a Clevenger-type distillation apparatus and a Dean-Stark distillation trap in a domestic microwave oven (Kendo MO-124, 2.5 GHz, 800 W), as described (Stashenko et al., 2004). The extracts were obtained from 40 g of dried leaves of each plant, macerated with 200 mL ethanol and left in suspension for seven days at 28 °C. The mixture was filtered and concentrated using a Buchi rotavapor. Stock solutions of 20 mg/mL for oils and 40 mg/mL for the extracts, were prepared in DMSO for subsequent bioassays.

Antifungal activity assays

The antifungal activity of the oils and extracts was evaluated following the Clinical and Laboratory Standards Institute M38-A (CLSI M38-A, 2002) protocol for filamentous fungi, and the standard method proposed by the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing (AFST-EUCAST) for yeasts (Cuenca-Estrella et al., 2003). *C. krusei* (ATCC 6258) and *A. fumigatus* (ATCC 204305) were used to evaluate antifungal activity. Briefly, duplicate 100 μL samples of five serial dilutions of the essential oils and extracts were dispensed into 96-well microtitration plates (Becton Dickinson, New Jersey, USA) at final concentrations between 31.25-500 μg/mL. Amphotericin B and itraconazole (Sigma-Aldrich, Co, MO, USA) were used as positive controls at final concentrations of 0.031-16 μg/mL. Tween 80 was included at a final concentration of 0.001% (v/v) to enhance oil solubility. One hundred microlitres of the fungal inoculum of 1-5 x 10^5 CFU/mL and 0.8 x 10^4-1x10^5 CFU/mL for yeast and filamentous fungi, respectively, were added. For the AFST-EUCAST method, the MIC was determined after 24 h of incubation at 35 °C and defined as the lowest concentration that resulted in 90% reduction of growth. For the CLSI M38-A method, the MIC were determined after 48 h of incubation at 35 °C, and defined as the lowest essential oil and extract dilution that resulted in total inhibition of visible growth. Essential oils and extracts were considered active when they presented MIC values ≤500 μg/mL. The MIC values were expressed as geometric means (GM-MIC) of tests performed in duplicate in the three different assays against each of the fungi species.

Interaction of essential oils and drugs

Assays were performed in flat-bottomed 96-well microtitration plates using the chequerboard method (Vitale et al. 2005). *C. krusei* was used at a final concentration of 0.5-2.5 x 10^5 CFU/mL. The final concentrations of the itraconazole and amphotericin B ranged from 0.004 to 2 μg/mL and the essential oil of *C. ambrosioides* L.from 0.488 to 31.25 μg/mL. The fractional inhibitory index (FICI) was calculated and the interpretation was determined as follow: ≤0.5 Synergistic effect; >0.5 but <4, no interaction; and ≥4 antagonistic effect (Vitale et al. 2005).

Time-kill assay

The *in vitro* pharmacodynamics of *C. ambrosioides* L. oil with *C. krusei* ATCC 6258 was performed as described by Klepser et al. (1998). One hundred microliters of an initial inoculum ranging from 1-5 x 10^5 CFU/mL was seeded onto flat-bottomed 96-well microtitration plates and 100 μL of each sample, at concentrations of 0.5, 1, 2 and 4x MIC, were added. The samples were incubated at 35 °C with agitation. At 0, 4, 8, 12, and 24 h, 10 μL were removed from each test solution for three serial dilutions (1:10). Volumes of 10 μL were then spread onto potato dextrose agar and incubated at 37 °C for 24 h to determine the number of CFU/mL. The limit of detection was 100 CFU/mL. Time-kill curves with itraconazole and amphotericin B served as fungistatic and fungicide controls, respectively. Experiments were carried out in duplicate in two separate experiments. Time-kill curves were constructed by plotting of mean±standard deviation (SD) of colony count ($\log_{10}$ CFU/mL) as a function of time (hours) with the statistical package Prisma (GraphPad Software, Inc., USA, 2007). Fungicidal activity was defined as ≥3 - $\log_{10}$ (≥99.9%) reduction in CFU/mL from the starting inoculum (0.5- 2.5 x 10^5 CFU/mL).

Cytotoxicity assay

Cercopithecus aethiops African green monkey kidney cells (Vero cell line ATCC CCL-81) were used. The cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% of fetal bovine serum (FBS), 100 units/mL of penicillin, 100 mg/mL of streptomycin, 20 mg/mL of L-glutamine, 0.14% $NaHCO_3$, and 1% each of nonessential amino acids and a vitamin solution. The cytotoxicity of the essential oils and their components was examined *in vitro* using an MTT (dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Sigma, New Jersey, USA) assay as described (Betancur-Galvis et al., 2002). Briefly, cells were plated at 1.4 x 10^5 cells/mL in 96-well flat-bottomed plates, and incubated at 37 ºC in a humidified incubator with 5% CO_2. After 24 h of incubation, each diluted oil or extract was added to the appropriate wells, and the plates were incubated for an additional 48 h at 37 ºC. Supernatants were removed from the wells, and 28 μL (2 mg/mL) of an MTT solution in MEM, supplemented with 10% FBS, were added to each well. Plates were incubated for 1.5 h at 37 ºC, and then 130 μL of DMSO were then added to dissolve the purple formazan crystals that were produced. The plates were placed on a shaker for 25 min, and absorbance was read at 550 nm on a multiwell spectrophotometer (Titertek Uniskan). The minimal dilution of the essential oils that induced 50% growth inhibition of the cells was expressed as IC50. The IC50 values for each compound were obtained by linear regression analysis of the dose-response curves generated from the absorbance data with the statistical

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package R (Development Core Team, Vienna, Austria, 2008). IC50 values were expressed as the Mean±SD of two independent experiments done in quadruplicate.

RESULTS AND DISCUSSION

MIC of 23 active samples (GM≤500 µg/mL), tested against *C. krusei* and *A. fumigatus* and the respective IC50 values are presented in Table 1. Six essential oils, and three extracts showed activity only against *C. krusei* (GM-MIC range 7.82 to 500 µg/mL), whereas four essential oils and two extracts were active only against *A. fumigatus* (GM-MIC range 125 to 500 µg/mL). Seven oils and one extract showed a major spectrum of activity because they were active against both strains. There is no agreement on the level of acceptance for plant products when compared with standards; therefore, some authors consider only activity comparable to antibiotics, while others consider even higher values (Hennebelle et al., 2008). According to

the classification of biological activity used by Holetz et al. (2002), the extract of *M. cucullata* (GM-MIC = 31.25 µg/mL) and the oil of *C. ambrosioides* L. (GM-MIC = 7.82 µg/mL) displayed strong activity against *C. krusei*. The activity of both samples was similar or higher than fluconazole when it was evaluated with ten clinical isolate of *C. krusei* (GM-MIC = 32 and 64 µg/mL) (Espinel-Ingroff et al., 2005). Additionally, MIC value obtained with *C. ambrosioides* oil was lower than the reported by Vasquez et al. (2000) for *Melaleuca alternifolia* oil (tea tree oil) with an isolate of *C. krusei* (8-16 µg/mL). *M. alternifolia* oral solution has been used as an natural topical antiseptic, furthermore it has showed efficacy for treatment of fluconazole-resistant oropharyngeal *Candida* infections in AIDS patients (Jandourek et al., 1998) and for treatment of vaginal candidiasis (Hammer et al., 1998)

In Colombia, the leaves, roots and flowers of *C. ambrosioides* have been approved as anthelmintic and antidiarrhea (Fonnegra & Jimenez, 2007). In the present

Table 1. Geometric Means of Minimal Inhibitory Concentration (GM-MIC) and Inhibitory Concentration 50 (IC50) of essential oils and extracts with antifungal activity.

Samples	GM-MIC (µg/mL)		IC50±SD (µg/mL)	
	C. krusei	*A. fumigatus*	Vero Cell line	R²
T. lucida (512074)*	>500	250	≥200	NA
P. racemosa (512227) *	>500	250	≥200	NA
N. acutifolia (520339)*	250	500	57.5±9.3	0.88
L. citriodora (484334) *	222.7	99.21	37.9±4.3	0.89
L. dulcis (512079) *	>500	125	≤25	NA
L. origanoides (512075) *	250	125	60.4±11.2	0.83
L. citriodora (480749) *	99.21	62.5	116.9±6.6	0.97
R. officinalis (531011) *	500	>500	53.6±11.4	0.80
H. racemosum-fruits (517005) *	396.85	>500	32.0±5.6	0.76
Hedyosmun sp. (517005) *	396.85	250	28.1±1.6	0.97
T. aff. *diffusa* Willd. Ex Schult. (516293)*	99.21	500	52.2±5.2	0.93
Hedyosmum scaberrium Standl. (517321)*	>500	500	≤25	NA
A. triphylla (517189)*	250	99,21	≥200	NA
C. ambrosioides L. (519592)*	7.82	>500	≥200	NA
B. graveolens (Kunth) Triana & Planch. (517753)*	500	>500	28.0±4.8	1.00
T. lucida (512074)*	500	>500	31.9±6.4	0.70
C. citratus (531013)*	314.98	>500	≥200	NA
M. royoc L. (512222)**	250	250	≥200	NA
E. cf. *uniflora* (512226) **	250	>500	117.4±11.9	0.92
M. cucullata (512228)**	31.25	>500	≥200	NA
S. amethystina J.E. Smith (521071) **	>500	500	≥200	NA
L. fucata Lindl. (521031)**	500	>500	≤25	NA
H. perbullata (521095) **	>500	250	≥200	NA
Amphotericin B	0.630	1.260	-	-
Itraconazole	0.125	0.157	-	-

*Essential oil; ** Extract; R²: linear regression coefficient; NA, Not applicable; The GM-MIC of actives essential oil and extract are indicated in bold.

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study, the essential oil of one plant of the same species, was not active against *A. fumigatus* (GM-MIC>500 μg/mL). However, the oil of the same plant evaluated with the poisoned food technique completely inhibited mycelial growth of this filamentous fungi at 100 μg/mL (Kumar et al., 2007). This difference in antifungal activity between essential oils from plants of the same species can be explained by the chemotype (chemical composition), geographical region of plant collection, age of plant, extraction method of oils, method used to assess the antifungal activity or by the intra or inter species differences (Samaranayake et al., 1995; Cruz et al., 2007; Maksimovic et al., 2008). Sharma & Tripathi (2008) have shown that the antifungal activity of essential oils is better evaluated in liquid than in solid media, since in the latter, the hydrophobic nature of the majority of essential oil components hamper uniform diffusion of these substances through the agar medium (de Cerqueira et al., 2007). We used the standard microdilution methods M38-A and AFST-EUCAST for the evaluation of antifungal susceptibility of filamentous fungi and yeasts, respectively. These methods make it possible to reduce the amount of oil used, to simultaneously evaluate several samples, and reproducibility in the results.

Chemical analyses of the oil of *C. ambrosioides* were previously carried out in our laboratory. The main component of the oil is the terpene ascaridole (31.0%) (unpublished data). The antifungal activity of this terpene has been previously demonstrated against *Aspergillus flavus, A. glaucus, A. ochraceous, Colletotrichum gloeosporioides, A. niger, C. musae* and *Fusarium oxysporum* (Jardim et al., 2008). It is possible that anti-*C. krusei* activity obtained in this study could be explained by a higher concentration of ascaridole.

H. perbullata is the extract that showed the best activity against *A. fumigatus* (GM-MIC = 250 μg/mL). This species is native of the Chicamocha canyon (Boyacá and Santander, Colombia), and is known as "Mastranto azul del Chicamocha" (Fernandez-Alonso, 2006). This is an aromatic plant whose antimicrobial activity as extract or essential oil has not been reported. However, essential oils of other species such as *H. suaveolens* and *H. ovalifolia* have antibacterial, anti-*C. albicans* and anti-dermatophyte activity (Asekun et al., 1999; Souza et al., 2003). Extracts (1) and oils (7) were active against both *C. krusei* and *A. fumigatus*; *L citriodora* was the most active (GM-MIC = 99.21 and 62.5 μg/mL, respectively). The antifungal activity of essential oils of various species of the *Lippia* genus have been reported but not of *L. citriodora* (Oliveira et al., 2007; Hennebelle et al., 2008). Chemical analyses of oil from *C. ambrosioide*s have also been carried out in our laboratory: the main components were geranial (18.9%) and neral (15.6%) (unpublished data). Both compounds were also the major compounds of the essential oil of *Thymus pannonicus* (41.42 and 29.61%, respectively) to show anti-*C. albicans* activity (Maksimovic et al., 2008); consequently, the anti-mycotic activity of *L. citriodora* can

result from the action of these terpenes. The only extract active against both strains (GM-MIC of 250 μg/mL) was that of *M. royo*c L. Extracts from other species such as *M. elliptica* and *M. angustifolia* displayed different biological activities, including antifungal (Xiang et al., 2008; Ali et al., 2000). Nevertheless, to our knowledge, the antifungal activity of *M. royoc* L has not been reported.

The criteria of cytotoxic activity for the crude extracts, as established by the American National Cancer Institute (USA), is an IC50<30 μg/mL (Suffness & Pezzuto, 1990). According to this criterion, the essential oils of *Hedyosmun* spp. and *L. dulcis*, were the only cytotoxic oils. This finding is very important given the need to find new antimycotic compounds with low-toxic effects.

To obtain oils and extracts active against *C. krusei* and *A. fumigatus* is important because *C. krusei* has been recognized as a potentially multidrug-resistant pathogen and *A. fumigatus* is the main cause of invasive aspergillosis in immunocompromised patients; moreover, only a limited number of drugs are available (Brock et al., 2008, Pfaller et al., 2008). Additionally, both *C. krusei* and *A. fumigatus*, have the ability to form biofilms in biomedical devices that come in contact with skin, mucosal or inert surfaces (Quindós et al, 2009; Seidler et al., 2008). Previous studies have reported the reduction of *C. albicans* biofilms by essential oils (Agarwal et al., 2008). Therefore, based on this information and our results, a possible application of these natural products could reside in the control of biofilm formation.

The time-kill dynamic process is used for the evaluation of new antimicrobial agents. This makes it possible to determine if the agent has a fungistatic or fungicidal effect, and if the killing process is concentration or time-dependent (Pfaller et al., 2004). Analysis of the time-kill curves of oil from *C. ambrosioides* L. with *C. krusei* did not have a significant effect at 0.5 x and 1 x MIC, whereas a fungicidal effect (CFU /mL >3 log units) was detected at 4 x MIC, after 4 h (Figure 1a). This behavior was comparable to the effect of amphotericin B (Figure 1b). Fungicidal activity is clinically more important than fungistatic activity, particularly in HIV patients, because prophylactic use of fungistatic drugs has been associated with an increased frequency of innate or acquired drug resistance in clinical isolates (Monk & Goffeau, 2008). At 1 x MIC there was a reduction in growth of UFC/mL until approximately 4 h, with renewed growth after 8 h of incubation. At 24 h the colony values were close to those of the growth controls. This analysis was made in comparison with the time-kill curve obtained with amphotericin B and itraconazole (Figure 1b and Figure 1c).

Potential synergy of essential oils with antibiotics has previously been considered with the aim of increasing the rate of fungal killing, shorten the duration of therapy, avoid the emergence of drug resistance, expand the spectrum of activity, and decrease drug-related toxicity by allowing lower doses of antifungal agents to be administered (Shin

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& Lim, 2004). Unfortunately, in this study an indifferent effect was found when oil of *C. ambrosioides* L. was combined with itraconazole or amphotericin B (FICI range = 1.03-1.06 and 1.03-1.00, respectively).

The studies carried out in our laboratory have shown that *C. krusei* is more susceptible to essential oils than *C. parapsilosis* (Mesa-Arango et al., 2007). Samaranayake et al. (1995) demonstrated that *C. krusei* is

more hydrophobic than other species. This physicochemical characteristic could at least partly explain the susceptibility of this yeast to the oils due to the hydrophobic nature of the majority of the components of the essential oil (de Cerqueira et al., 2007).

In conclusion, the essential oil of *L. citriodora* was the sample with the broadest spectrum of activity. Moreover the essential oil of *C. ambrosioides* and
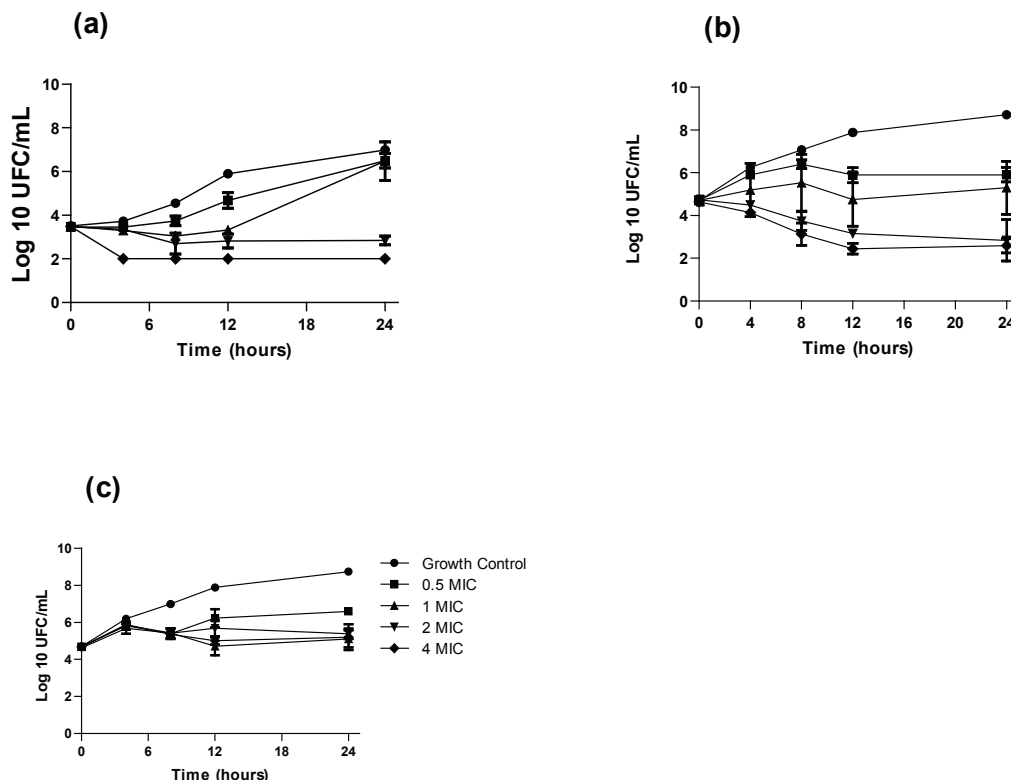
(a)

(b)

(c)

Figure 1. Time-kill plots of (a) *C. ambrosioides* L. oil, (b) amphotericin B and (c) itraconazole against *C. krusei* ATCC 6258. ● Growth control; ■, 0.5 x MIC; ▲, 1 x MIC; ▼, 2 x MIC; ♦, 4 x MIC.

the extract of *M. cucullat*a inhibited *C. krusei in vitro*, at similar concentrations as fluconazole. In addition, the samples were not cytotoxic. The results presented suggest that these oils and extracts could constitute promising candidates for the development of new antifungal agents and should stimulate studies on toxicity, improved formulations and the determination of optimal concentrations for clinical applications, as well as on comparative studies alongside currently used drugs.

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