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Antifungal activity of natural compounds against *Candida* species isolated from HIV-positive patients

Débora Oro[1], Andréia Heissler[1], Eliandra Mirlei Rossi[1,2], Diane Scapin[1], Patrícia da Silva Malheiros[2*], Everton Boff[1]

[1]*Microbiological Research and Diagnostic Laboratory, Department of Biological and Health Sciences, University of Western Santa Catarina Sao Miguel Oeste, SC, Brazil*

[2]*Hygiene and Food Microbiology Laboratory, Department of Food Sciences, Food Science and Technology Institute, Federal University of Rio Grande do Sul, ICTA/UFRGS, Porto Alegre, RS, Brazil*

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ABSTRACT

Objective: To evaluate the antifungal effect of *Cinnamomum zeylanicum* (*C. zeylanicum*) and *Melaleuca alternifolia* essential oils and honey against strains of *Candida* sp. from HIV-positive patients in order to subsidize new therapeutic strategies for candidiasis.

Methods: The study evaluated the antifungal effect of natural antimicrobials against 30 strains of *Candida* sp. isolated from oral cavities in HIV-infected patients. Then, they were compared to the action of fluconazole and amphotericin B. Antifungal susceptibility was evaluated by the broth macrodilution technique and the minimum inhibitory concentration and the minimum fungicidal concentration were determined.

Results: Among all antifungals evaluated in this study, amphotericin B was the one showing the best results; however, all compounds studied here showed inhibitory activities against isolates of *Candida* sp. Honey (0.0313–64 μg/mL) demonstrated fungistatic activity inhibiting 70% of the isolates. *C. zeylanicum* essential oil (0.0313–64 μg/mL) inhibited 93.3% of the *Candida* strains and *Melaleuca alternifolia* essential oil (0.0313–64 μg/mL) was able to inhibit 73.3% of them.

Conclusions: Therefore, all natural compounds evaluated in this study, especially *C. zeylanicum* essential oil, may become promising agents for oral candidiasis therapy including in HIV-positive patients.

1. Introduction

Oral candidiasis is the most common clinical manifestation in patients infected with HIV, being a sign of immune imbalance that usually heralds the transition to AIDS. In these patients, *Candida albicans* (*C. albicans*) is the species responsible for most infections [1–4].

The increased resistance to available antifungal agents and the toxicity contained in some of these drugs has reduced the effectiveness of treatments [2,4,5]. Therefore, it is very important for the discovery of new antifungal agents, in order to widen the spectrum of activity against *Candida* and fight strains resistant to conventional antifungal drugs such as amphotericin B and fluconazole.

In this context, many natural products have shown to be alternative therapies for the treatment of candidiasis [5,6]. Among these natural products, essential oils are known to have antimicrobial properties; however, only in recent years the science has elucidated the action mechanism of these compounds.

Melaleuca alternifolia (*M. alternifolia*), a native plant from Australia and commonly known as tea tree oil, and *Cinnamomum zeylanicum* (*C. zeylanicum*), a plant found in Sri Lanka and southern parts of India, have both shown potential antimicrobial action against a wide variety of bacteria and fungi [7,8].

*Corresponding author: Patrícia da Silva Malheiros, Hygiene and Food Microbiology Laboratory, Department of Food Sciences, Food Science and Technology Institute, Federal University of Rio Grande do Sul, ICTA/UFRGS, Porto Alegre, RS, Brazil.
Tel: +55 513308 7861
E-mail: patricia.malheiros@ufrgs.br

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Besides its essential oils, honey has been recognised around the world for its anti-microbial properties [9]. Researches have shown the antifungal potential of honey against *Candida* sp. [10]. However, just few ones have been made studying the inhibition of *Candida* strains isolated from HIV-infected patients.

In this context, the objective of this study was to evaluate the antifungal effects of *C. zeylanicum and M. alternifolia* essential oils and honey against strains of *Candida* sp. from HIV-positive patients in order to subsidize new therapeutic strategies for candidiasis.

2. Materials and methods

2.1. C. albicans isolates

The study isolated 30 strains of *Candida* sp. from the oral cavity of HIV-infected patients. These yeasts were subjected to the germ tube technique identifying presumptive species of *C. albicans.* For the tube germ test, a colony of each strain was transferred to another one containing 0.5 mL of 1% peptone water, which was incubated at 37 °C for 2–3 h. After that, the suspension was examined under optical microscope (1 000×) in order to visualize the germ tube formation.

2.2. Inoculum

The inoculum was prepared from 48-h cultures in Sabouraud dextrose agar supplemented with chloramphenicol (Difco, France), stopping up the fungal cells in sterile distilled water. The cell density was adjusted with a spectrophotometer (Biospectro) at wavelength of 530 nm in order to obtain the transmittance of 0.5 MacFarland scale. Thereafter, the inoculum was diluted in RPMI 1640 (Sigma Chemical, St. Louis), which resulted in a final concentration of 2.5×10^3 cells/mL^2.

2.3. Essential oils, honey and antifungal drugs

Essential oils were purchased from the Petite Marie Fine Chemicals LTD company (Itaquaquecetuba-SP). Honey was purchased in a supermarket (Sulmel®, Xaxim-SC) and it was used in pure form. All the composts were diluted in RPMI 1640 medium (Sigma Chemical) in order to offer final concentrations from 0.031 3 μg/mL to 64 μg/mL.

The suspension of amphotericin B and fluconazole were prepared according to the protocol M27-A2 [11]. The final concentrations for amphotericin B ranged from 0.031 3 μg/mL to 16 μg/mL and, for the fluconazole, ranged from 0.125 μg/mL to 64 μg/mL.

2.4. Susceptibility tests

The minimum inhibitory concentration (MIC) was performed through broth macro-dilution technique [11]. For this, 100 μL aliquots were added to each concentration of test substance (honey and essential oils or antifungal drugs) and 100 μL of inoculum in sterile test tubes, which were incubated at 36 °C for 24–48 h. All tests were performed in triplicate.

The minimum fungicidal concentration (MFC) determination was obtained by transferring 100 μL of each concentration that inhibited the fungal growth to the surface of Sabouraud agar supplemented with chloramphenicol (Difco). After incubating them for 48 h, the MFC was defined as the lowest concentration of drug capable of causing the death to 100% of fungal cells. This was demonstrated by the absence of colonies in plate [11].

According to the Clinical And Laboratory Standards Institute [11], the strains is sensitive to fluconazole if MIC $\leq$ 8 μg/mL, intermediate or susceptible dose-dependent to MIC from 16 to 32 μg/mL and it is resistant to MIC $\geq$ 64 μg/mL. Breakpoints have not been established for amphotericin B during the interpretation of results. However, according to data from the Clinical and Laboratory Standards Institute [11], when MIC > 1 μg/mL for *Candida* sp., it is likely that the isolate is resistant to this drug.

2.5. Statistical analysis

Data on inhibitory effects of essential oils, honey, fluconazole and amphotericin B on *Candida* spp. growth were subjected to analysis of variance (ANOVA) and paired *t*-tests, using SPSS statistical program, version 17.0. A significant difference when $P < 0.05$ was considered for these tests.

3. Results

In this study, from 30 *Candida* strains collected from oropharyngeal HIV-positive patients, 22 (73.3%) of them showed positive in germ tube test, indicating the presence of *C. albicans.*

The amphotericin B demonstrated greater effectiveness than fluconazole inhibiting 100% of the evaluated *Candida* strains at least in one of the concentrations (0.031 3–16 μg/mL), and the fungicidal effect was observed in 93.3% of the samples. Fluconazole also inhibited 100% of *Candida* strains at least in one of the concentrations (0.125–64 μg/mL). However, the fungicidal effect was seen in only 20% of the samples.

In this sense, other alternatives are necessary to control candidiase infection such as the use of essential oils. *C. zeylanicum* essential oil (0.031 3–64 μg/mL) inhibited 93.3% of the *Candida* strains evaluated in this study and *M. alternifolia* essential oil (0.031 3–64 μg/mL) was able to inhibit 73.3%. Nevertheless, the fungicidal effect of essential oils was less than the fungistatic one showing percentages of 76.7% for *C. zeylanicum* and 36.7% for *M. alternifolia.*

The honey (0.031 3–64 μg/mL) was able of inhibit 70% of *Candida* isolates, although it showed no fungicidal effects (Table 1), and its fungistatic effect was lower compared to both assessed essential oils.

Table 1

Average from MIC and MFC of the studied compounds against non-*C. albicans* (μg/mL).

Substance	MIC	MFC
Fluconazole	5.80 ± 0.80	13.33 ± 1.00
Amphotericin B	0.62 ± 0.50	6.04 ± 0.80
C. zeylanicum	3.39 ± 0.60	8.88 ± 0.90
M. alternifolia	4.84 ± 0.50	32.00 ± 1.00
Honey	8.04 ± 0.90	NFE

NFE: No fungicide effect.

Table 2
Average from MIC and MFC of the studied compounds against *C. albicans* (μg/mL).

Substance	MIC	MFC
Fluconazole	3.26 ± 0.80	42.67 ± 0.90
Amphotericin B	0.66 ± 0.30	6.61 ± 0.80
C. zeylanicum	10.45 ± 1.00	16.43 ± 1.20
M. alternifolia	20.03 ± 0.50	25.33 ± 0.80
Honey	14.25 ± 0.80	NFE

NFE: No fungicide effect.

Table 2 shows that the average MIC of the *C. zeylanicum* essential oil was of 10.45 μg/mL to *C. albicans.* Also using *C. zeylanicum* essential oil, Unlu *et al.* found higher MIC values, *i.e.* of 0.07 mg/mL and 1.12 mg/mL for two *C. albicans* strains [12], demonstrating the high variability of sensitivity between these different strains.

To *M. alternifolia* essential oil, it was found average MIC of 20.03 μg/mL to *C. albicans* (Table 2) and 4.84 μg/mL to non-*C. albicans* (Table 1). Rosato *et al.* found a MIC between 1.75 and 3.5 mg/mL in *M. alternifolia* oil to different *Candida* strains [13] and Thosar *et al.* found a MIC of 0.5 μL/mL to *C. albicans* strains using the same oil [14].

4. Discussion

Oral candidiasis is an opportunistic infection commonly found in HIV-positive patients. It leads to reduction in quality of life and increased mortality. *C. albicans* has been shown as a widespread species in HIV/AIDS patients [1,3]. The findings of this work show that although the frequency of non-*C. albicans* species is increasing, *C. albicans* remains the most prevalent species in several cities of the world [1].

Amphotericin B and fluconazole are important drugs used in the candidiasis treatment. Fluconazole is usually the first choice drug for systemic infection; however, studies have shown the increase of *Candida* strains resistant to this drug [1]. Others works also found that *C. albicans* isolates were more sensible to amphotericin B than fluconazole [2], including HIV-infected patients [15].

The differences between the fungicidal effect of amphotericin B and fluconazole may be caused by their distinct action mechanisms. While amphotericin B action is based on the formation of pores in the fungal cell membrane, altering permeability becomes excellent to destroy the cell, and fluconazole inhibits the biosynthesis of ergosterol, an essential component of fungal cell plasma membrane, causing only the infeasibility of this cell [2]. Furthermore, the indiscriminate use of fluconazole may have contributed to the increased number of strains resistant to this antifungal.

Besides, the results found in this study confirm that the antifungal properties assigned to honey make it a promising agent for the treatment of oral candidiasis [10,16]. These results are important, especially if compared to other ones in which the MIC ranged from 38% to 46% [10,17].

The MIC results shown in Tables 1 and 2 allow us to note that the yeast classified as non-*C. albicans* species had mean a MIC lower than *C. albicans,* except for the fluconazole. These data demonstrate that non-*C. albicans* strains are more sensitive to the tested substances when compared to *C. albicans.* Overall, the average MFC was also lower for non-*C. albicans* than for *C. albicans.* Although, such results can be influenced by the number of samples, it has been found greater amount of *C. albicans* (22 strains).

Comparing all antifungals studied here, it was possible to note that amphotericin B showed the best performance against the *Candida* isolates. Fluconazole showed better effects than *M. alternifolia* oil; nevertheless, its fungistatic action was similar to *C. zeylanicum* essential oil and honey. Among the evaluated essential oils, *C. zeylanicum* showed significantly better effects when compared to *M. alternifolia* oil.

The results also showed that amphotericin B is an excellent antifungal to control *Candida* sp., since it showed the best results among all compounds studied. However, the toxicity attributed to this drug limits its use, thus, it is necessary to apply alternative therapies when assisting patients in the treatment of candidiasis. Thus, the synergistic effect of some essential oils, such as *M. alternifolia,* can be an interesting alternative when used in combination with amphotericin B [13].

Accordingly, the studied compounds showed a significant antifungal activity against *Candida* sp., strengthening the role of folk medicine during the treatment of candidiasis. This demonstrates the feasibility of using these compounds as adjuvants for the treatment of oral candidiasis, especially in HIV patients, making them as promising for the treatment of this type of fungal infection.

All evaluated natural compounds, *i.e. C. zeylanicum* and *M. alternifolia* essential oil and honey, showed antifungal effects against *Candida* species isolated from HIV-positive patients. Moreover, the *C. zeylanicum* essential oil has proven to have higher antifungal activity and it needs further investigation to evaluate its feasibility in clinical applications for the treatment of superficial candidiasis, especially in HIV-positive patients.

Conflict of interest statement

We declare that we have no conflict of interest.

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