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Research Article

Anti-Candida Activity of the Essential Oil From *Elionurus Muticus*: A Preliminary Study

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Abstract

Background: Candida albicans, a commensal fungus, develops as a resilient pathogen under the condition of host dysbiosis. In recent decades, research on medicinal plants has been an alternative in the discovery of new biocomponents for various applications in folk medicine. The genus *Elionurus* contains antiseptic, sudoriferous and febrile properties.

Aim: To evaluate the *in vitro* antimicrobial activity of *Elionurus muticus* essential oil on the *Candida* spp.

Materials and Methods: The determination of the Minimum Inhibitory Concentration (MIC) by the serial microdilution procedure. The minimum fungicidal concentration was determined by SDA plating.

Results: Data from the MIC tests showed values of 0.0312 to 0.125mg/ml and MFC values of 0.0625 to 0.5 mg/ml.

Conclusions: The essential oil of *Elionurus muticus* is biologically active against the Candida samples tested. The essential oil of this species has fungicidal activity against most of the strains tested, with the exception of *C. parapsilosis* CBS 604, *C. krusei* CBS 573 and *C. utilis* CBS 5609.

Keywords: *Candida;* Antifungal; Oil essential

Introduction

Candida species are found as commensal microorganisms in the gastrointestinal and reproductive organs in most healthy individuals but may become pathogenic when host defenses are breached or under the condition of microbial dysbiosis. Although the most frequently isolated species are *C. albicans* in the last decade, the number of infections caused by non-albicans species has increased significantly. More than 90% of the cases of invasive infection by *Candida* are attributed to five species: *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis* and *C. krusei* [1,2].

Therapeutic options for the treatment of candidiasis are mainly azole, polyene and echinocandin type antibiotics. Unfortunately, the clinical use of these agents has been limited due to their toxicity and resistance factors, contributing to high rates of morbidity and mortality [3].

In recent decades, research on medicinal plants has been an alternative in the discovery of new biocomponents for various applications in folk medicine. The use of essential oils extracted from different types of plants, showed several indications, as antiseptic, antifungal and antibacterial actions [4].

The genus *Elionurus* presents approximately 45 species. It occurs in Africa, Asia, North America and South America. *Elionurus muticus* is a grass of the Brazilian pampas known as Brazilian lemongrass. This has aroused considerable interest in the phytochemical industry. It presents antioxidant activity related to the content of its phenolic compounds. It is notable for containing antiseptic, sudoriferous and febrile properties. Produces essential oil in all its extension: in the aerial part, leaves and inflorescences, as in the roots. However, both the composition and the yield are variable according to the region of cultivation and the seasonality. Despite this, this essential oil may be rich in citral, which is a mixture of two geometric isomers known as geranians and nerals. Some pharmacological properties of citral have been reported in the literature, including antitumor, bronchodilator, antiprotozoal and antimicrobial effects [5,6].

Based on the literature data, the objective of this work was to evaluate the *in vitro* antimicrobial activity of *Elionurus muticus* essential oil on the *Candida* spp. Strains by determining the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (CFM).

Material and Methods

Essential oil

The essential oil of *Elionurus muticus* was commercially acquired from the company "Harmonia Natural", Canelinha - SC, Brazil.

Reference strains

The strains of Candida used were: *C. albicans* (ATCC 90028, ATCC 2876 and CBS 562), *C. guilhermondii* (CBS 566), *C. krusei* (CBS 573), *C. utilis* (CBS 5609), *C. rugosa* (IZ 12), *C. lusitaniae* (IZ 06), *C. glabrata* (IZ 07), *C. dubliniensis* (CBS 7987), *C. parapsilosis* (CBS 604).

Antifungal activity of essential oils

Minimum Inhibitory Concentration (MIC): Samples of the essential oils were tested for their activity on *Candida* spp strains determining the Minimum Inhibitory Concentration (MIC) by the

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Table 1: Minimal Inhibitory and Minimum Fungicide Concentration of *Elionurus muticus* essential oil.

MICRORGANISMS	MIC	MFC	MFC/MIC	OIL ACTIVITY
C. albicans ATCC 90028	0,125 mg/mL	0,25 mg/mL	2	Fungicide
C.albicans ATCC 2876	0,125 mg/mL	0,25 mg/mL	2	Fungicide
C. guilhermondii CBS 566	0,0625 mg/mL	0,125 mg/mL	2	Fungicide
C.krusei CBS 573	0,0312 mg/mL	0,125 mg/mL	4	Fungistatic
C. utilis CBS 5609	0,0312 mg/mL	0,125 mg/mL	4	Fungistatic
C. rugosa IZ 12	0,0312 mg/mL	0,0625 mg/mL	2	Fungicide
C. lusitaniae IZ 06	0,25 mg/mL	0,25 mg/mL	1	Fungicide
C. glabrata IZ 07	0,125 mg/mL	0,25 mg/mL	2	Fungicide
C. dubliniensis CBS 7987	0,125 mg/mL	0,125 mg/mL	1	Fungicide
C. albicans CBS 562	0,125 mg/mlL	0,25 mg/mL	2	Fungicide
C. parapsilosis CBS 604	0,0625 mg/mL	0,5 mg/mL	8	Fungistatic

MIC = Minimal Inhibitory Concentration; MFC = Minimal Fungicidal Concentration

M27-A3 protocol recommendations [7].

Inoculum adjustment: It was prepared in saline solution, equivalent to $5.0x10^6$ CFU/mL in spectrophotometer. The inoculum was standardized at $2.5x10^3$ by serial dilution.

In a sterile microplate, 100 µl of RPMI was distributed in all wells, the first column added 100 µl of the essential oil at the initial concentration, and then the serial microdilution of the essential oils was carried out. After the dilution process was added 100 µl of the adjusted inoculum, giving the following groups: a) Positive control group: culture medium and inoculum, for observation of yeast growth); b) Negative control group 1: culture medium only, for observation possible contamination; c) Negative control group 2: culture medium, essential oil and diluent, the purpose of observing possible contaminations in the microdilution); d) Negative control group 3: culture medium and diluent, for the purpose to observe possible toxic effects of the diluent on the yeast; e) Test group: *Elionurus latiflorus* essential oil (2mg/ml initial concentration) or antifungal treatment commercial Fluconazole (initial concentration 64 µg/mL) or Amphotericin B (Initial concentration 16 µg/mL). The plates were incubated for 24 hours at 37°C in an aerobiose oven, and visual reading was performed. The tests were performed in triplicate.

Determination of minimum fungicidal concentration – MFC

The determination of the Minimum Fungicidal Concentration (MFC) was made in Sabouraud Dextrose Agar plates - SDA (KASVI). The 96 wells were homogenized by pipetting, and an aliquot of 10 µl was transferred to the Petri dish. After incubation at 37°C for 48h, the lowest fungicidal concentration was established. MFC was determined as the lowest concentration of essential oils, which does not allow the growth of any fungal colony in solid culture through visual reading to confirm the fungicidal and fungistatic effects of the essential oils tested [8]. The assays were performed in triplicate.

Results

The essential oil was tested against reference strains of *Candida* spp. in order to determine the minimum inhibitory concentration by broth microdilution technique. The data demonstrated inhibitory activity at concentrations of 0.0312mg/mL to 0.125mg/mL for the essential oil of *Elionurus muticus*, as shown in Table 1.

The essential oil was tested against reference strains of *Candida* spp. in order to determine the minimum inhibitory concentration by broth microdilution technique. The data demonstrated inhibitory activity at concentrations of 0.0312mg/mL to 0.125mg/mL for the essential oil of *Elionurus muticus*, as shown in Table 1.

After determination of the MIC, an aliquot of the susceptibility assay was used to determine the Minimum Fungicidal Concentration (CFM) against the strains of *Candida* spp. *E. muticus* showed better activity at concentrations ranging from 0.0625mg/mL to 0.500mg/mL, as can be seen in Table 1.

The essential oil of Elionurus muticus showed fungistatic activity for *C. parapsilosis* CBS 604, *C. krusei* CBS 573 and *C. utilis* CBS 5609 with MIC and MFC values: 0.0312 and 0.125mg/ml; 0.312 and 1.25mg/ml; 0.0625 and 0.500mg/ml, respectively. The fungicidal activity was detected for *C. albicans* ATCC 90028, *C. albicans* ATCC 2876, *C. guilhermondii* CBS 566, *C. rugosa* IZ 12, *C. lusitaniae* IZ 06, *C. glabrata* IZ 07, *C. dubliniensis* CBS 7987, *C. albicans* CBS 562, *C. parapsilosis* CBS 604: that presented smaller variation between the relation CFM and MIC.

Discussion

In recent decades, research on medicinal plants has been a viable resource in the discovery of new drugs for various applications in folk medicine. The use of essential oils extracted from several general of plants, presented several therapeutic indications, among them the antiseptic, antifungal and antimicrobial action [4].

Elionurus muticus is a grass of the Brazilian pampas. This has aroused considerable interest in the phytochemical industry. It is notable for containing antiseptic, sudoriferous and febrile properties [5,6].

Data from the MIC tests showed values of 0.0312 to 0.125mg/ml and MFC values of 0.0625 to 0.5mg/ml. These results demonstrate antifungal activity of the essential oil of *Elionurus muticus* against the Candida spp strains tested.

Results in the literature also describe the antifungal activity of E.muticus essential oil against Candida albicans, C. krusei and Cryptococcus neoformans with MIC values ranging from 0.0005mg/ml to 0.005mg/ml and CFM ranging from 0.0025 to 0.01mg/ml, using a diffusion disc technique, a methodology different from that used in this research [9].

These suposed divergences suggest that factors such as: differences in applied techniques, in the strains used and in the growing regions of *Elionurus muticus* may be contributing factors in obtaining non-similar data [5,10-12].

The antimicrobial activity of the essential oil of *Elionurus muticus* is also confirmed in some articles using the technique of microdilution in broth and disc diffusion, obtaining values of MIC (Minimal Inhibitory Concentration) that range from 1mg/ml to 150mg/ml against *Escherichia coli, Staphylococcus aureus, Coagulase-negative*

Staphylococcus, Staphylococcus pseudintermedius, Streptococcus uberis, Pseudomonas aeruginosa and *Bacillus cereus* [13-15].

The results presented in this research, demonstrated the effectiveness of the essential oil of *Elionurus muticus* in low concentrations on several microbial strains, corroborating with the data of the literature [15,9].

These data, when analyzed together, indicate, despite the different methodologies employed, that the *E. muticus* species may be a viable alternative in the search for new biocomponents present in the plants with antimicrobial potential, since complementary data of the chromatographic profile of more species, soil types, seasonality and methodologies of the OE extraction, are obtained, increasing the knowledge of these medicinal plants.

Ongoing studies based on these considerations and the evaluation of the activity of *Elionurus muticus* oil in planktonic cells of *Candida* spp. in the biofilm of *Candida albicans* SC5314, associated with *in vitro* cytotoxicity tests in HaCat cells and *in vivo* in *Galleria mellonella* larvae, are being carried out with the objective of increasing the knowledge of this essential oil as an alternative of antifungal and antimicrobial action as substituents or as adjuvants of synthetic drugs in the treatment of microbial diseases.

Conclusion

The essential oil of Elionurus muticus is biologically active against the Candida strains tested.

The essential oil of this species has fungicidal activity against most of the strains tested, with the exception of *C. parapsilosis* CBS 604, *C. krusei* CBS 573 and *C. utilis* CBS 5609.

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