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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Evaluation of anti-enzyme properties of *Origanum vulgare* essential oil against oral *Candida albicans*

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Summary This study aimed to evaluate the anti-enzymatic activity of *Origanum vulgare* (oregano) essential oil against 15 strains of *Candida albicans*. *Candida albicans* samples were isolated from the oral mucosa of patients with denture stomatitis treated in a Dentistry school on a public university. Preparation of the inoculum was performed with a suspension of *C. albicans* reactivated 24 h earlier in 5 mL of sterile phosphate buffer saline (PBS) adjusted to a 0.5-turbidity on the MacFarland scale ($1{,}5 \times 10^8$ UFC/mL). The essential oil was obtained by hydrodistillation in a Clevenger-type machine and analyzed by gas chromatography. Enzymatic assay was performed to test phospholipase anti-enzymatic properties. Chromatography analysis revealed that the main compounds present in the essential oil were 4-terpineol (41.17%), thymol (21.95%), γ-terpinene (5.91%) and carvacrol (4.71%). For the anti-enzymatic test, the statistical analysis showed that there was found statistically significant interactions between the factors time and concentration ($P \leq 0{,}001$). Thus, essential oil of oregano at 1%, 5% and 10% presented significant reductions in the production of the phospholipase enzyme produced by *Candida albicans* strains. However, the longer the incubation time of the essential oil, there is a relatively moderate reduction in its anti-enzymatic activity.
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Introduction

The use of plants in the treatment and cure of diseases is as old as the human species itself. However, even today in the poorest regions of the country and in the major Brazilian cities, medicinal plants are commercialized in open markets, popular stores and is found even in many residence backyards. The plants of the family Lamiaceae have aroused interest due to their potential as an antimicrobial agent, where many of the species of this family, introduced in Brazil, are medicinal plants and producers of essential oils, being also used as condiments and ornamental flowers as the *Origanum vulgare*.

The essential oil of *Origanum vulgare* has demonstrated good bactericidal and fungicidal activity against different pathogens, being attributed to the compounds carvacrol and thymol, which are the phenolic components present in great quantity in some essential oils, like those present in the *Origanum vulgare [ref]*. Therefore, there is a growing interest in the use of essential oils, due to their antioxidant and antimicrobial properties. Although the use of this material occurs in greater quantities in the food industry, it has also been used in pharmaceutical industries because of its therapeutic properties. Furthermore, *Origanum vulgare* has shown good satisfactory inhibition properties. According to Shivraj [1] it revealed a 82% inhibition percentage towards xanthine oxidase.

Among the various opportunistic pathogenic microorganisms, *Candida albicans* has been considered as the most frequent yeast in oral infections. This fact leads several clinicians and researchers to seek methods to inhibit or attenuate this type of infection [2]. Interactions between yeasts of the genus *Candida* and host cells are essential for colonization, tissue invasion and onset of disease. These yeasts use a series of devices, known as virulence factors [2]. The *C. albicans* species uses three main mechanisms for pathogenicity and invasion, such as the escape of immune system responses [3]; the morphogenic change from yeast to forms of hyphae, which increases the ability of yeast to adhere to and invade host cells [4]; and the invasion of host cells, which is supported by factors associated with hyphae, such as adhesion molecules [5], invasion-like molecules [6] and secreted hydrolytic enzymes [7].

Candida albicans is an exclusive producer of one of the major hydrolytic enzymes produced, the phospholipases [8], which is an essential enzyme in the adherence of this fungus to the cell wall [9]. Also, this kind of enzyme attacks the common phospholipids of any cell membrane and are associated with the ability of the yeast to adhere to host epithelial tissue.

In view of the aforementioned, the present work aimed to evaluate the antifungal capacity of oregano essential oil, including its anti-enzymatic properties tested against exoenzymes produced by *Candida albicans* isolates.

Materials and methods

Essential oil

To obtain the oil, 100 g of *Origanum vulgare* samples were weighed and hydrodistilled in a Clevenger type apparatus, according to the Brazilian Pharmacopoeia IV, for 5 h. Aerial parts of *O. vulgare* was acquired with botanical certifications and originated from Chile (Luar Sul(r) — Indústria e Comércio de Produtos Alimentícios Ltda., Santa Cruz do Sul, RS, Brazil).

After extraction, the oil was dried over anhydrous Na_2SO4, P.A., concentrated under ultrapure nitrogen (N2) and stored in amber flasks, and kept under refrigeration until analysis. The yield of the *O. vulgare* oil was 0.4 mL/100 g.

Chromatography analysis

The essential oils obtained were analyzed by gas chromatography with flame ionization detector (GC/FID-Schimadzu 17A), equipped with a DB-5 silica column (30 m $\times$ 0.25 mm $\times$ 0.25 μm), with an initial temperature of 40 °C, with an increase in the 2 °C min^{-1} until reaching 145 °C. From this temperature, the rate was 10 °C min^{-1} until reaching 280 °C, remaining at that temperature for 10 min; Td = 280 °C; Tinj = 280 °C; Tcol = 40 °C; Split = 1:50. Solutions (5,000 μg mL^{-1} in hexane) of the essential oils were prepared and 1 μL of these solutions were injected into the chromatograph. A solution (40 μg mL^{-1}) of the chromatographic standards (a-pinene, camphene, b-pinene, myrcene, a-terpinene, p-cymene, limonene, 1,8-cineole, a-terpinene, terpinolene, Linalool, 4-terpineol, α-terpineol, thymol and carvacrol), which was subjected to the same conditions as the samples. The compounds in the samples were identified by comparison with the terpene retention time standards and with data from the literature. Data were acquired through the EZChrom Elite Compact(r) program (Agilent(r)).

Culture medium

The phospholipase medium was prepared by adding 13 g of Sabouraud Agar, 11.7 g of crystallized NaCl and 0.11 g of CaCl in 180 mL of distilled water [10]. The solution was cooked at 121 °C, without boiling until acquire a dark and translucent colour.

The sterile medium was cooled in a water bath to a temperature of about 45 °C without hardening, with 7.2 g of pure egg yolk being added at that time. After addition of the egg yolk, the phospholipase medium was deposited in Petri dishes.

Anti-phospholipase activity test

The study was performed according to the methods used by Kadir [10], using 15 strains of *C. albicans* isolated from patients with prosthetic stomatitis at the Oral Microbiology Laboratory of a Federal University, and the samples were processed in triplicate.

For the test, *Origanum vulgare* oil (''oregano'') was diluted at 1%, 5% and 10% in Dimethylsulfoxide (DMSO) and inoculum preparation was performed with a *C. albicans* suspension, reactivated 24 hours before the test on a Sabouraud Agar culture with a 37 °C temperature, in 5 mL of sterile Saline Phosphate Buffer (PBS) to 0.5 turbidity on the MacFarland scale (1.5 $\times$ 108 CFU/mL).

Then, the essential oil was diluted to 0.1; 0.5 and 1.0% in sterile PBS (20 μL in 1.98 mL PBS) and added to 0.5 mL of the inoculum.

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