# Evaluation of the inhibition of strains of Candida Albicans by the essential oil of *origanum vulgare*

# Avaliação da inibição de cepas de Candida Albicans pelo óleo essencial de *Origanum vulgare*

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## ABSTRACT

*Candida albicans* is a yeast normally found in the human microbiota, but it is considered opportunistic, causing a pathology called candidiasis. The essential oil of oregano (*Origanum vulgare*) has thymol and carvacrol in its composition, compounds with proven antimicrobial and antifungal action. Thus, the present study evaluated the Minimum Inhibitory Concentration (MIC) of essential oil of oregano on the strain ATCC 90028 of *Candida albicans* through macro and microdilution techniques, where it showed antifungal activity in vitro. Fungal suspensions were treated with different concentrations

of essential oil (100, 50, 25, 12.5, 6.25, 3.125 and 1.56 uL/mL) where it was possible to identify the MIC's of 6.250 uL/mL and 12.5 uL /mL in the macrodilution and microdilution techniques, respectively. Thus, it is concluded that the essential oil of *Origanum vulgare* has a promising antifungal action, especially on *Candida albicans*, and, therefore, potential therapeutic action.

**Keywords:** Candida Albicans, oregano essential oil, MIC.

**RESUMO**

Candida albicans é uma levedura normalmente encontrada na microbiota humana, mas é considerada oportunista, causando uma patologia chamada candidíase. O óleo essencial de orégano (Origanum vulgare) tem timol e carvacrol em sua composição, compostos com ação antimicrobiana e antifúngica comprovada. Assim, o presente estudo avaliou a Concentração Mínima Inibitória (MIC) de óleo essencial de orégano sobre a cepa ATCC 90028 de Candida albicans através de técnicas de macro e microdiluição, onde mostrou atividade antifúngica in vitro. As suspensões fúngicas foram tratadas com diferentes concentrações de óleo essencial (100, 50, 25, 12,5, 6,25, 3,125 e 1,56 uL/mL) onde foi possível identificar os MIC's de 6.250 uL/mL e 12,5 uL/mL nas técnicas de macro e microdiluição, respectivamente. Assim, conclui-se que o óleo essencial de Origanum vulgare tem uma ação antifúngica promissora, especialmente em Candida albicans, e, portanto, uma ação terapêutica potencial.

**Palavras-chave:** Candida Albicans, óleo essencial de orégano, MIC.

## 1 INTRODUCTION

*Candida albicans* is a microorganism present in the human microbiota, being present in different regions of the body, it is harmless to healthy individuals with immunological competence. However, it is one of the main fungal pathogens that cause infections, resulting from its rapid proliferation when there is an imbalance in the body and the immune system. There has been an increase in reports of infections caused by different species of the genus *Candida* in the last three decades, associated with infections by the *Human Immunodeficiency Virus* - HIV (GULATI and NOBILE, 2016; SOUZA; MORAIS; BARROS, 2022).

The oral pathology generated in newborns by *Candida albicans* has been reported in history for many years, with reports from Hippocrates in ancient Greece in the middle of the 5th and 4th century BC. The first clear and precise description of the etiological agent as a fungus was made in 1853 by Charles Philippe Robin, who named it ``*Oidium albicans*``. Later, in the 20th century, mycologist Christine Marie Berkhout fully described the genus *Candida*. Both terms come from the Latin “white, the color of light, clear and colorless” (LEDERMANN, 2017).

*C. albicans* is a fungus with a diploid genome with 14.3 megabases and eight chromosomes. The high genetic plasticity presented, provided by the support to a diversity of mutational processes, favors its ability to colonize different niches and adapt to different selective pressures, which is another factor that contributes to its resistance to antifungals. Species of the genus Candida have an accelerated adaptation capacity, mainly in the hematogenous environment, thanks to their mechanisms of adaptation to hostile environments, which results in high mortality rates, mainly in hospital environments. This way, another mechanism presented is biofilms, which by definition are a community of cells adhered to a surface or present at the air-liquid interface, wrapped in an extracellular matrix. Its main characteristic is resistance to chemical and physical injuries and, therefore, it is a structure that confers resistance to microorganisms. (CHANDRA; KUHN; GHANNOUM, 2001; NOBILE and JOHNSON, 2015; GULATI and NOBILE, 2016; ENE; BENNETT; ANDERSON, 2019; DOS SANTOS et al., 2022).

The use of plants with medicinal characteristics has been an alternative supported by the World Health Organization (WHO) and whose efficiency is described in the literature. Plants with medicinal properties have been used in different ways and described as an option for the treatment of various pathologies. With the increasing resistance of microorganisms to antifungal drugs, herbal medicines have become a very promising therapeutic option. Essential oils are extracted from aromatic plants and are mixtures of various chemical components, and several of these have antifungal activity established in the literature (MOLINA et al., 2008; VASCONCELOS, 2013).

In view of the emergence of resistance of *Candida albicans* to its main conventional treatments, the azoles, in addition to the great hepatotoxicity presented by the polyenes, the use of phytoalexins has become an interesting alternative, despite the fact that molecules derived from plants have a weaker antibiotic activity. The essential oil of *Origanum vulgare* contains phenols such as thymol, eugenol and carvacrol in its composition, compounds capable of causing cell damage and, therefore, with antimicrobial activity (CHAMI et al., 2004; ARAÚJO and LONGO, 2016).

Thymol (thymic acid) is a substance belonging to the terpene group and present in several plant species. This substance is described with antimicrobial and disinfectant pharmacological activity, and it is possible to find in the literature several descriptions of its in vitro antifungal action. Thymol interacts with *Candida albicans*, altering its adhesion and metabolic activity, acting on newly formed or mature biofilms. In view of

the biochemical nature of monoterpenes, it is likely that it acts as a cell wall solvent and makes it porous (VASCONCELOS, 2013).

Brito et al. (2015) used in their studies the essential oil of *Lippia sidoides* and Thymol against strains of *Candida albicans*, *Candida tropicalis* and *Candida krusei*, and found a great antifungal potential in these compounds, with the presence of morphological changes and inhibition of the formation of hyphae and pseudohyphae (BRITO et al., 2015).

Vasconcelos (2013) found in his essays that when using thymol as an antifungal it was possible to inhibit biofilm formation with a decrease in viable cells. Braga et al. (2008) demonstrated that thymol interferes in the first stages of biofilm development, with a large reduction in metabolic activity (BRAGA et al., 2008; VASCONCELOS, 2013).

Carvacrol and eugenol are other compounds present in the composition of oregano essential oil, in which inhibitory and antifungal action is also used. Chami et al. (2004) were able to demonstrate through their assays that eugenol and carvacrol were able to considerably reduce the colony forming units (CFU) and even eradicate the oral infection of *C. albicans* in rats (CHAMIS et al., 2004)

The importance of seeking to understand the virulence mechanisms of *C. albicans* is notorious, especially when related to strains that are resistant. Considering that this microorganism is one of the main causes of nosocomial infections, it is of great clinical relevance to seek alternatives for the treatment of affected patients and also efficient ways to combat the resistance conferred by it.

## 2 MATERIAL AND METHODS

The study was carried out at the Center for Microbiological and Agronomic Studies (CEMA) of the University Center of Southern Minas Gerais (UNIS - MG). A strain of *Candida albicans* from a licensed reference culture, American Type Culture Collection (ATCC) 90028, was used.

*Origanum vulgare* essential oil 5 ml is marketed by the company “By Samia” and was purchased in a store specialized in natural products. The same is steam distilled through the fresh or dried flowers of the plant, according to the manufacturer.

To test the sensitivity of the strains, the methodology described by Araújo and Longo (2016) and Molina et al. (2008) was adapted, in addition to following the recommendations established in the M27-A2 standard of the CLSI (Clinical and

Laboratory Standards Institute). The strains were grown in Sabouraud Dextrose medium and incubated at 37ºC for 48 hours in a bacteriological oven. After the incubation period, a suspension was prepared in Brain Heart Infusion broth (BHI) and in Tryptone Soy broth (TSB), and these suspensions had their turbidity adjusted to 0.5 on the McFarland scale, which corresponds to $1.5x10^8$. To prepare the oil, it was diluted in concentrations of $10^1$, $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$. This serial dilution was also performed in BHI and TSB broths.

Macrodilution was performed in micro tubes containing 0.9 ml of the broth with different dilutions of the essential oil and 0.1 ml of the fungal suspension adjusted to 0.5 on the McFarland scale. A positive control tube was made, containing only 0.9 ml of broth without the addition of essential oil and 0.1 ml of fungal suspension. For the negative control, a tube with 0.9 ml of the broth with the highest dilution of the essential oil (100 uL/mL) was used. The microtubes were incubated at 37ºC for 24 hours in a bacteriological oven and after this period they were visually observed to verify turbidity.

To determine the Minimum Inhibitory Concentration (MIC) of oregano essential oil, microdilution in sterile 24-well plates was used. For this, a technique similar to that used in microtubes was used, with serial dilution and a positive control and a negative control. The plate was incubated at 37ºC for 24 hours and after this period the results were observed under the microscope with 10x and 40x objectives and compared with the control wells.

In addition, after obtaining the data, they were recorded and analyzed using descriptive statistics, using percentages, graphs and tables, as well as using analysis of variance (ANOVA).

## 3 RESULTS

According to the manufacturer of the essential oil of *Origanum vulgare*, when performing gas chromatography, approximately 9.90% of thymol and up to 80% of terpineol and carvacrol were found in the composition, in addition to smaller percentages of monoterpene and sesquiterpene hydrocarbons, phenolic acids, flavonoids, among others. It is important to point out that the concentrations of the components may vary according to the geographical location, type of planting and harvest phase of the plant.

Among the seven dilutions made for the essential oil of oregano, in each of these it is possible to find the following concentrations: 100 uL/ml, 50 uL/ml, 25 uL/ml, 12.5 uL/ml, 6.25 uL/ ml, 3.125 uL/ml, 1.56 uL/ml. This standard of dilutions was used for the macro and microdilution test. Those samples in which the presence of the essential oil

inhibited *Candida albicans* cell multiplication were considered sensitive, and those in which there was growth were considered resistant.

The results demonstrate that *Origanum vulgare* oil was capable of inhibiting the growth of the ATCC 90028 strain. The MIC found for the essential oil was 6.250 uL/mL in the macrodilution test and 12.5 uL/mL for the microdilution test, values that are shown in table 1.

Table 1. Results of the antifungal activity tests of the essential oil of Origanum vulgare by the microdilution and macrodilution technique for standard samples (ATCC 90028) of Candida albicans.

***Candida albicans ATCC 90028***

| | *CIM (uL/mL) 1* | *CIM (uL/mL) 2* | *AVERAGE* | *VARIANCE* |
|---|---|---|---|---|
| **MICRODILUTION** | 12,5 | 6,25 | 9,375 | 19,53125 |
| **MACRODILUTION** | 12,5 | 25 | 18,75 | 78,125 |

Source: The authors, 2022

A variance of 19.53 was found for the microdilution technique at a significance level of 0.01, considering the two broths as repetition. For the macrodilution technique, a variance of 78.12 was found. Bearing in mind that the treatments have means within the significance level, it is concluded that the values are statistically equal.

After the incubation period, in the samples that were resistant, it was not possible to identify fungal growth either through turbidity or through microscopy.

## 4 DISCUSSION

The use of plants and compounds with medicinal properties for various purposes is a popular practice widespread in society, even before the development of studies that elucidate these medicinal mechanisms. It is well known that the antibacterial activity of several essential oils and their compounds is described in the literature, but the same does not occur for the antifungal activity. Plant species of the *Lamiaceae* family have proven antifungal activity and great therapeutic potential. The essential oil of *Origanum vulgare* is relevant because it has high stability, diversity of compounds and demonstrated antifungal action for several species of yeast (VIANA, 2013).

Braga et al. (2008) demonstrated in their analyzes that the isolated use of Thymol, a compound present in the composition of the essential oil of oregano, was capable of interfering in the stages of biofilm production in ATCC strains of *Candida albicans* (BRAGA et al., 2008).

Lambert et al. (2001) investigated in their studies the antimicrobial capacity of oregano essential oil compared to isolated thymol and carvacrol compounds. From this it was possible to conclude that the combined effect of thymol and carvacrol are responsible for the inhibition found in the essential oil of oregano, since it was evidenced in the analyzes carried out that the destructive effects of membrane permeability correlated with similar inhibition profiles for *Staphylococcus aureus* and *Pseudomonas aeruginosa* (LAMBERT et al., 2001).

In the present study, it was not possible to perform chromatography to determine the compounds present in the essential oil used and their concentrations, as is done in several similar studies in the literature. Viana (2013) when performing the chromatography of the essential oil of oregano found a predominance of the constituent Carvacrol over Thymol and other compounds. Cleff et al. (2013), when analyzing eight essential oils of oregano from different origins, concluded that each of them had a different thymol/carvacrol ratio. With this in mind, it is possible to observe that the essential oil of the same plant can actually present different constitutions, depending on the way of manufacture and extraction, in addition to the type of planting and harvesting. This fact justifies the difference found in MIC values in similar studies using *Origanum vulgare* essential oil, considering that its constituents and proportions are important factors for its performance as an antifungal agent (CLEFF et al., 2013; VIANA, 2013).

Cleff et al. (2013) observed the presence in the literature of great variation in the composition of the essential oil of oregano and correlated the variations in the MIC values with the different concentrations and combinations of thymol and carvacrol, and this variable is directly related to the efficiency antifungal and antimicrobial activity of the oil (CLEFF et al., 2013).

Cleff and his study group (2010) also analyzed the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of oregano essential oil for *Candida albicans*. A value of 2.97 uL/ml was found for MIC and 3.54 uL/ml for CFM, in reference culture strains. The study showed differences in susceptibility between clinical strains and reference strains. This indicates that the differences in the MIC values found between the present study and other descriptions in the literature, can be associated with the differences found between different strains, reference or not, of the yeast and also differences between essential oils with different concentrations of the presented compounds, in addition to the differences presented between the techniques (CLEFF et. al, 2010).

Lambert et al. (2001) point out that when using solvent, detergent and emulsifier together with essential oil, a technique widely used in several studies, the presented MIC values are higher than when compared to methodologies that do not use these compounds. This indicates that these compounds decrease the action of the antimicrobial. None of these agents were used in the present study, and therefore, it is important to observe this parameter when comparing the results obtained with those found in the literature (LAMBERT et al., 2001).

The antimicrobial action employed by the oil is related to the mechanism of increased membrane permeability, changes in cytoplasmic pH and leakage of intracellular ions. It is also possible to observe damage related to nutrient absorption, nucleic acid synthesis and ATPase activity. It is observed that the components present in the essential oil are capable of harming the cell membrane and allowing access to the genetic material of the microorganism (LAMBERT et al., 2001).

Bona et al. (2016) when analyzing the action of several essential oils against 30 clinical strains of *Candida albicans* and the ATCC 14053 strain, found that the most effective were mint, winter savory and oregano. In addition, they were also able to identify that the aforementioned oils were effective in 100% of the analyzed strains. Therefore, the literature confirms the great effectiveness of the essential oil of *Origanum vulgare* specifically against *Candida albicans*, an effectiveness that is maintained even with the variability presented among the different strains (BONA et al., 2016).

Another important point is that the variance presented between the analyzed methods can be related to the broths of different compositions used in the repetitions, however it demonstrates a small variability of the effectiveness of the oil in vitro.

The results show that even with the difference shown between strains or between the concentrations of essential oils, which proportionally generates variability in the MIC, the essential oil of Origanum vulgare and its compounds are promising against yeasts, showing inhibition at concentrations considered low.

## 5 CONCLUSION

Therefore, oregano essential oil has an excellent inhibitory activity against *Candida albicans* strains, as previously described in the literature. With this, it is concluded that treatment strategies using these compounds and the essential oil should be considered and analyzed by health professionals, researchers and the industry, in order to obtain a new treatment mechanism and even to circumvent the resistance presented by

microorganisms. The present study had the limitation of not performing the chromatography of the essential oil and, therefore, it was not possible to determine with specificity the amount of each compound found. In addition, to obtain a more detailed study, it is important to carry out tests in order to define the Minimum Fungicide Concentration (MFC). Furthermore, it is important to consider the present study as fundamental for the development of other studies with different approaches. For example, to try to elucidate the interaction of essential oil compounds with the biofilm formed by *Candida albicans.*

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