

## Short Research Article

# Maximum inhibitory dilution of a water suspension of fresh garlic extract against oral yeasts of *Candida albicans* isolated from patients hospitalized in an Intensive Care Unit

## ABSTRACT

**Aims:** To determine the maximum inhibitory dilution (MID) of a water suspension of against oral yeasts of *Candida albicans* isolated from patients hospitalized in an Intensive Care Unit (ICU).

**Study design:** This is a Cross-sectional study used to oral *Candida* collected from hospitalized patients in Intensive Care Unit.

**Place and Duration of Study:** Sample: Oral yeasts of *Candida albicans* isolated from patients in Intensive Care Unit (ICU) of the *Hospital das Clínicas* (HC - hospital school) of the Universidade Federal de Goiás (UFG) (ICU/HC/UFG).

**Methodology:** – A bulb of around 5 g of fresh garlic was peeled and crushed in a sterile beaker; 0.16 g of the garlic mass obtained was diluted in 100 mL of sterile water and left to soak from 6 to 8 hours and covered in a laminar flow hood. After manual homogenization of the garlic and water solution and filtration, 10 serial dilutions from 1/2 to 1/1024 (800 to 1.56 µg/mL) were made in sterile test tubes containing 5 mL of Sabouraud dextrose broth (SDB) with the addition of chloramphenicol. Suspensions in autoclaved water, McFarland standard no. 3, of each sample of *Candida* yeasts from the oral cavity of 90 patients hospitalized in an ICU were prepared and 1 mL of homogenized *Candida* solution added to each test tube with its respective dilution. The test tubes were kept at a temperature of 37°C/24 h. The reading of the MID of each oral sample of *Candida albicans* in the presence of garlic water homogenized in SDB resulted from macroscopic observation of the first maximum dilution in which there was not turbidity of the *Candida* isolate evaluated.

**Results:** All the oral cultures of *Candida albicans* were sensitive to the MID of 1/32 of garlic water solution.

**Conclusion:** The garlic water suspension proved capable of inhibiting in vitro growth of oral yeasts of *Candida albicans* of patients from a hospital ICU, showing potential fungistatic action in relation to the fungus evaluated.

**Keywords:** *Candida albicans*, garlic, mouth

## Introduction

Garlic is a bulbous and medicinal herbaceous plant known since antiquity, originating in Central Asia [1]. This monocotyledonous herb with a strong aroma and flavor, used throughout history in cooking, dates back in therapeutic applications from Eastern civilizations: Greek, Egyptian, Indian, and Chinese [1]. It is a plant of the Liliaceae (lily) family (*Allium sativum* L.), and an asexual plant that is propagated through planting of its cloves [1,2]. Variability of different types of garlic is known, and nearly all differ in size, color, shape and flavor, number of cloves in the bulb, acidity, and storability [3].

Biochemically, the garlic bulb has from 0.04 to 0.37% sulfur in the forms of diallyl disulfide, diallyl trisulfide, and S-Allyl-L cysteine sulfoxide (alliin), as well as other volatile compounds (linalool, geraniol,

and citral), enzymes, minerals, lipids, and proteins. When garlic cloves are chopped up, allicin (diallyl s-oxide) is obtained from its biologically inactive precursor (alliin). Allicin and thiosulfate are the main bioactive compounds of garlic that have antimycotic activity. These chemical substances act in destructuring the fungal cell and inducing fungicide action [4].

High levels of *Candida* yeast are detected in the oral microbiota of patients hospitalized in intensive care units (ICU), which favors a pathogenic process by the fungus [7,8]. Pseudomembranous candidiasis is the main fungal infection brought about by *Candida* yeasts that are present in the oral microbiota [5]. Among the *Candida* species pathogenic to humans, *Candida albicans* is the mycological agent most detected. The highly infectious nature of *Candida albicans* isolates is due to marked manifestation of virulence factors in pathogenic processes [6-7]. Adherence, morphological dimorphism, phenotypic variability or switching, enzymes (aspartyl proteinases and phospholypases), and toxins (toxic glycoproteins and candidotoxins) are biochemical and physiological abilities of *Candida* yeasts that are expressed when the fungus exercises the role of opportunistic agent [5-7].

The increased frequency of oral isolates of *Candida* resistant to the antifungal derivatives available on the market has stimulated the search for new therapeutic alternatives. Phytotherapy has proven to be a promising and viable pharmacological field for development of new natural medications with effectiveness against yeasts of the *Candida* genus.

The aim of this study was to detect the maximum inhibitory dilution in water suspension of fresh garlic extract against oral yeasts of *Candida albicans* isolated from patients hospitalized in an ICU.

## Methodology

### *Candida isolates*

Isolates were obtained from 90 samples of oral isolates of *Candida albicans* from patients hospitalized in the ICU in the city of Goiânia, GO, Brazil. These yeast cultures were kept at room temperature in the fungus collection of the *Laboratório de Análises Microbiológicas em Saúde* of the *Instituto de Patologia Tropical e Saúde Pública* of the Universidade Federal de Goiás (LAMSA/IPTSP/UFG).

The project which gave rise to this study obtained the approval of the ethics committee of the *Hospital das Clínicas* of the Universidade Federal de Goiás (HC/UFG) under protocol (CEPMHA/HC/UFG) no. 634.432/2014 and followed all the directives for research on human beings.

### *Reisolation and reactivation of virulence capacity*

The yellowish-white colonies of *Candida albicans* from the oral microbiota of individuals in the hospital ICU and kept in the fungal collection, with passage every two months, were once more cultured in Sabouraud dextrose broth (SDB) with the addition of chloramphenicol and enriched with inactivated human serum at 5%. After being kept in a laboratory oven at 37°C/24h, 2 mL of each *Candida*

suspension, manually homogenized, was seeded onto Petri dishes with Sabouraud dextrose agar (SDA) with the addition of chloramphenicol. After 24 h more, the colonies of oral cultured *Candida albicans* were transferred with a sterile inoculation loop to test tubes, previously identified, containing DAS with antibiotic, and then kept once more in the fungus collection at room temperature.

#### Maximum Inhibitory Dilution (MID)

A bulb of around 5 g of fresh garlic was peeled and crushed in a sterile beaker; 0.16 g of the garlic mass obtained was diluted in 100 mL of sterile water and left to soak from 6 to 8 hours and covered in a laminar flow hood. After manual homogenization of the garlic and water solution and filtration, 10 serial dilutions from 1/2 to 1/1024 (800 to 1.56 µg/mL) were made in sterile test tubes containing 5 mL of SDB with the addition of chloramphenicol. Suspensions in autoclaved water, McFarland standard no. 3, of each sample of *Candida* yeasts from the oral cavity of 90 patients hospitalized in an ICU were prepared and 1 mL of homogenized *Candida* solution added to each test tube with its respective dilution. The test tubes were kept at a temperature of 37°C/24 h. The reading of the MID of each oral sample of *Candida albicans* in the presence of garlic water homogenized in SDB resulted from macroscopic observation of the first maximum dilution in which there was not turbidity of the *Candida* isolate evaluated.

## Results and discussion

All the cultures of oral *Candida albicans* from patients hospitalized in the ICU proved to be sensitive in vitro to a MID of 1/32 of fresh garlic extract solution in water (Table 1).

### Insert table

The fungistatic action of the water suspension of fresh garlic extract on the oral yeasts of *Candida albicans* from patients in a hospital ICU was observed in vitro with an MID oscillation variation from 1/2 (800) to 1/32 (50 µg/mL) (Table 1). This was compatible with the study of Meriga et al. (2012)[10] in the Asia Pacific region in which an aqueous solution of garlic showed antifungal activity against the yeasts of *Candida albicans* in a variation of concentration from 150 to 100 µg/mL, representing effectiveness of 79.4% in relation to the dilutions of the water suspension of fresh garlic extract used in our study against oral isolates of *Candida albicans* from patients hospitalized in a hospital ICU. Even so, 68.9% of the cultures of *Candida albicans* from the oral cavity of individuals placed in a hospital ICU continued more susceptible to an MID of 1/32 of garlic water suspension, equivalent to a concentration of 50 µg/mL (Table 1). Allicin, one of the main biochemical compounds of garlic with antimycotic action, proved to be effective in vitro against the proliferation of yeasts of *Candida albicans* used in relation to the triazole medication used, such as fluconazole [8]. This antifungal action of allicin extended to inhibition of the ability for morphological yeast-like dimorphism to the filamentous form, necessary for pathogenicity of the fungus and induction of the lack of formation of biofilm through suppression of expression of the HWP1

gene detected through comparative analyses of molecular biology [09]. However, an Iranian study carried out by Khodavandi et al. (2011) [10] showed that allicin also interferes in the expression of the SIR2 gene at a potentiality two times greater than the minimum inhibitory concentration exercised by fluconazole in relation to gene inhibitory action. In addition, allicin, upon blocking the significance of the SIR2 gene, suppresses the ability of morphological variability of *Candida* yeasts during the occurrence of the infectious process. However, it remains inert in expressiveness of the SAP1-4 genes, related to production of the aspartyl proteinases exoenzyme that acts in destructuring the mosaic fluid of the cytoplasmic membrane of the cell of the parasite infected host. However fluconazole inhibits expression of the SAP4 gene by fungistatic action, but through an alternative biochemical pathway[10,11]. The technique of the drug diffusion method in agar, through the use of disks resulting from antibiotic association with fresh garlic extract, showed an increase in pharmacological effectiveness over 60 Chinese multiresistant samples of methicillin resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* in relation to the 30 likewise multiresistant isolates of *Pseudomonas aeruginosa*[12] Morphogenesis and extracellular fungal enzymatic yield are two other yeast-like characteristics that undergo suppressive interference from the bioactive compounds of diallyl sulfide (DS) and diallyl disulfide (DADS)[15] present in fresh garlic extract. In New Delhi, India, Yousuf et al. (2012)[13] showed that the minimum inhibitory concentration (MIC) of DADS and DS were 500 µg/mL and 40 µg/mL, respectively, for the clinical isolate used; and 450 µg/mL and 50 µg/mL, respectively, for the reference isolate ATCC 980028. Half of the MIC of DADS and DS inhibited the production of proteinases in 35% and 24%, respectively, in the clinical isolate used. In the ATCC strain, it was 44% and 28%, respectively. In relation to productivity of phospholipases, DADS and DS inhibited 60% and 27%, respectively, when used on the clinical sample, and 64% and 31% in use of the reference strain. For induction of hypha formation of *Candida* in 5 h, it was observed that it was 15% at half of MIC of DS, and 5% also at half of MIC of DADS, in comparison to the control used, which was 90%. When the clinical sample was used, detection of hypha was 16% in the presence of half MIC of DS and 8% in half MIC of DADS, in relation to 95% in the control. However, the Chinese study by Li et al. (2016) [14], analyzing antifungal activity and kinetic and molecular mechanism in the case of garlic oil in relation to *Candida albicans* yeasts, showed that MIC of 0.35 µg/mL of garlic oil can have a lethal effect on the yeast fungus used. This oil, moreover, has the ability of penetrating the cell membrane of *Candida albicans*, as well as the membranes of the cytoplasmic organelles, such as mitochondria, resulting in their destruction and, finally, fungal cell apoptosis. Analysis of the RNA sequencing of *Candida albicans* is revealing regarding induction of differential expression of critical genes, including those involved in processes of redox, pathogenesis, and cell response to drugs. Enzymatic functions present in the fungus cell, mediated by the putative cytoplasmic adenylate kinase, pyruvate decarboxylase, hexokinase, and thermal shock proteins also seem to be suppressed by the action of garlic oil [14] Continuity of the antifungal action attributed to garlic extract is still connected with the sulfur action exercised by allicin. In the first decade of the 2000s, Jennifer et al (2005) [15] already showed the efficacy of fresh garlic extract on planktonic and sessile

cells present in the biofilm induced by *Candida* yeasts and that antifungal activity in vitro decreases as the phenotype of biofilm develops, as previously observed in traditional antifungal drugs. An in vitro synergistic effect was also observed between the use of allicin and azole derivatives – ketoconazole and fluconazole. Only the Malaysian species of *Candida*: *albicans*, *glabata*, and *tropicalis* were susceptible to this synergistic association [16]. A randomized Iranian clinical trial with 40 patients with prosthetic candidiasis showed the equivalence of treatment effectiveness with aqueous garlic extract or nystatin in recovery of erythematous oral lesions[17]. For their part, Gruhlke et al. (2010) [18] acknowledged that antimicrobial activity of allicin results from oxidative inactivation of essential enzymes containing thiol. This substance in aqueous suspension of garlic can activate the apoptosis of yeast-like cells through its oxidizing properties, and this is a mechanism that kills the cells through a pathway that is an alternative to specific oxidizing inactivation previously proposed of essential enzymes. Furthermore, allicin interacts synergistically in vitro and in vivo with amphotericin B [19] increasing the oxidizing capacity of the polyene antibiotic on isolates of *Candida albicans*. An et al. (2009) [19] foresee that a combination between amphotericin B and allicin may come to be a promising strategy for therapy of disseminated candidiasis. Garlic, as a medicinal plant in the forms presented (fresh extract, powder state, and oil), has been used throughout the world, especially in the Far East, for centuries, and scientifically corroborates its effective pharmacological action not only against fungal infections, but also in relation to other pathologies brought about by viruses, bacteria, and parasites [20].

## Conclusion

Water suspension of fresh garlic extract at the MID of 1/32 (50µg/mL) proved to be able to inhibit proliferation in vitro of oral yeasts of *Candida albicans* originating from patients hospitalized in an ICU. This laboratory proof showed the possibility of using garlic in future therapeutic procedures of alleviation or cure for individuals frequently attacked by oral candidiasis to those needing clinical care in a hospital ICU.

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 226 Discov. 2010; 5(1): 91-3.

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229 **Table 1. Oral isolates of *Candida albicans*, originating from patients in a hospital ICU susceptible**  
 230 **to the maximum inhibitory dilution of fresh garlic extract in the presence of autoclaved water**

| Dilutions used/Cc of fresh garlic extract in SDB (µg/mL) | Oral isolates of <i>Candida albicans</i> susceptible |
|----------------------------------------------------------|------------------------------------------------------|
|                                                          | n = 90 (%)                                           |
| 1/2 (800)                                                | 89 (98.9)                                            |
| 1/4 (400)                                                | 82 (91.1)                                            |
| 1/8 (200)                                                | 78 (86.7)                                            |
| 1/16 (100)                                               | 65 (72.2)                                            |
| 1/32 (50)                                                | 62 (68.9)                                            |
| ≥ 1/64 (25)                                              | 00 (00.0)                                            |

231 Cc – Concentration of the medication  
 232 SDB- Sabouraud dextrose broth