International Journal of TROPICAL DISEASE & Health

25(3): 1-6, 2017; Article no.IJTDH.35874
ISSN: 2278–1005, NLM ID: 101632866

Maximum Inhibitory Dilution of Water Suspension Garlic Extract against oral *Candida albicans* Isolated from Patients Hospitalized in Intensive Care Unit

Evandro Leão Ribeiro[1], Flávia Liara Massaroto Cessel Chagas[1], Maria de Lourdes Breseguelo[1], Géssica Viviane de Oliveira[1], Lorranny de Jesus Gomes[1], Adriano Luis Fernandes Crocetti[1] and Clever Gomes Cardoso[1*]

[1]Laboratory of Medical Microbiological Analysis, Institute of Tropical Pathology and Public Health, Federal University of Goiás, Goiânia, GO, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ELR and CGC designed the study and wrote the first draft of the manuscript. Authors MLB and FLMCC wrote the protocol, managed the analyses of the study, revised first draft of the manuscript and managed the literature searches. Authors GVO, LJG, ALFC and ELR performed the experiments. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2017/35874
Editor(s):
(1) Ashlesha Kaushik, Pediatric Infectious Diseases, Unity Point Health, USA.
Reviewers:
(1) Fellah Mamoun, Khenchela University, Algeria and Annaba University, Algeria.
(2) Mustapha Umar, Nigerian Institute of Leather and Science Technology, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/20694

Short Research Article

Received 31st July 2017
Accepted 23rd August 2017
Published 28th August 2017

ABSTRACT

Aims: To determine the maximum inhibitory dilution (MID) of a water suspension of garlic extract 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 determine the effect of garlic extract against oral Candida collected from hospitalized patients in ICU.
Place and Duration of Study: Sample: Oral yeasts of *Candida albicans* isolated from patients in ICU of the *Hospital das Clínicas* (HC - hospital school) of the Universidade Federal de Goiás (UFG) (ICU/HC/UFG).

**Corresponding author: Email: evandro0@terra.com.br, clevergomes@gmail.com;*

Methodology: A bulb of 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.

1. 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 Unit (ICU), which favors a pathogenic process by the fungus [5]. 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 canditoxins) 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 [8].

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.

2. METHODOLOGY

2.1 Isolation and Identification of *Candida albicans*

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.

2.1.1 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 hours incubation, the colonies of *Candida albicans* were transferred to a sterile inoculation loop to test tubes, previously identified, containing SDA with antibiotic, and then kept once more in the fungus collection at room temperature.

2.2 Maximum Inhibitory Dilution (MID)

A bulb of 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 [9].

3. 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).

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. [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

Table 1. Maximum inhibitory dilution of fresh garlic extract against the isolated *Candida albicans*

Dilutions used/Cc of fresh garlic extract in SDB (µg/mL)	Oral isolates of *Candida albicans* 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)

Cc – Concentration of the medication, SDB- Sabouraud dextrose broth

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, such as fluconazole [11]. 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 [12,10]. However, an Iranian study carried out by Khodavandi et al. [13] 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 [13,14]. 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* [15] 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) [16] present in fresh garlic extract. In New Delhi, India, Yousuf et al. [16] 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 ATTC 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 ATTC 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. [17], 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 [17] 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. [18] 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 [19]. 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 [20]. For their part, Gruhlke et al. [21] 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 [22] increasing the oxidizing capacity of the polyene antibiotic on isolates of *Candida albicans.* An et al. [22] 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 [23].

4. CONCLUSION

In conclusion, the results presented herein demonstrate that water suspension of fresh garlic extract can inhibit proliferation in vitro of oral yeasts of *Candida albicans* originating from patients hospitalized in an ICU from a dose of 1/2 (800 µg/mL) to maximal inhibitory dilution of 1/32 (50 µg/mL). Further studies are needed, however our data are good indications that fresh garlic extract can be used as a natural source for the treatment of oral *Candida albicans* infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Leyla B, Peir HK, Ali G. Garlic: A review of potential therapeutic effects. Avicenna Jounal of Phytomedicine. 2014;4(1):1-14.
2. Lanzotti V, Barile E, Antignani V, Bonanomi G, Scala F. Antifungal saponins from bulbs of garlic, *Allium sativum* L. var. Voghiera. Phytochemistry. 2012;78:126-134.
3. Iciek M, Kwiecień I, Włodek L. Biological properties of garlic and garlic-derived organosulfur compounds. Environ Mol Mutagen. 2009;50:247-265.
4. Martins N, Petropoulos S, Ferreira IC. Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review. Food Chem. 2016; 15(211):41-50.
5. Sakaguchi H. Treatment and prevention of oral candidiasis in elderly patients. Med Mycol J. 2017;58(2):J43-J49.
6. Tamura NK, Negri FMN, Bonassoli LA, Svidzinski TIE. Virulence factors for *Candida* spp recovered from intravascular catheters and hospital workers' hands. Rev. Soc. Bras. Med. Trop. 2007;40(1): 91-3.
7. Bergsson G, Arnfinnsson J, Steingrímsson O, Thormar H. *In vitro* killing of *Candida albicans* by fatty acids and monoglycerides. Antimicrob Agents Chemother. 2001;45(11):3209-12.
8. Casaroto AR, Lara VS. Phytomedicines for Candida-associated denture stomatitis. Fitoterapia. 2010;81(5):323-8.
9. André RF, Andrade IM, Silva-Lovato CH, Paranhos HdeF, Pimenta FC, Ito IY. Prevalence of mutans streptococci isolated from complete dentures and their susceptibiligy to mouthrinses. Braz Dent J. 2011;22(1):62-7.
10. Wu X, Santos RR, Fink-Gremmels J. Analyzing the antibacterial effects of foods ingredientes: Model experiments with allicin and garlic extracts on biofilm formation and viability of *Staphylococcus epidermidis*. Food Sci Nutr. 2015;3(2): 158-68.
11. Meriga B, Mopuri R, MuraliKrishna T. Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum*. Asian Pac J Trop Med. 2012;5(5):391-5.
12. Khodavandi A, Harmal NS, Alizadeh F, Scully OJ, Sidik SM, Othman F, Sekawi Z, Ng KP, Chong PP. Comparison between allicin and fluconazole in *Candida albicans* biofilm inhibition and in suppression of HWP1 gene expression. Phytomedicine. 2011;19(1):56-63.
13. Khodavandi A, Alizadeh F, Harmal NS, Sidik SM, Othman F, Sekawi Z, Chong PP. Expression analysis of SIR2 and SAPs1-4

gene expression in *Candida albicans* treated with allicin compared to fluconazole. Trop Biomed. 2011;28(3): 589-98.
14. Low CF, Chong PP, Yong PV, Lim CS, Ahmad Z, Othman F. Inhibition of hyphae formation and SIR2 expression in *Candida albicans* treated with fresh *Allium sativum* (garlic) extract. J Appl Microbiol. 2008; 105(6):2169-77.
15. Li G, Ma X, Deng L, Zhao X, Wei Y, Gao Z, Jia J, Xu J, Sun C. Fresh garlic extract enhances the antimicrobial activities of antibiotics on resistant strains *in vitro*. Jundishapur J Microbiol. 2015;8(5): e14814.
16. Yousuf S, Ahmad A, Khan A, Manzoor N, Khan LA. Effect of garlic-derived allyl sulphides on morphogenesis and hydrolytic enzyme secretion in *Candida albicans*. Med Mycol. 2011;49(4):444-8.
17. Li WR, Shi QS, Dai HQ, Liang Q, Xie XB, Huang XM, Zhao GZ, Zhang LX. Antifungal activity, kinetics and molecular mechanism of action of garlic oil against *Candida albicans*. Sci Rep. 2016;7(6):22805.
18. Jennifer A, Shuford JA, Steckelberg JM, Patel R. Effects of fresh garlic extract on *Candida albicans* biofilms. Antimicrob Agents Chemother. 2005;49(1):473-7.
19. Khodavandi A, Alizadeh F, Aala F, Sekawi Z, Chong PP. *In vitro* investigation of antifungal activity of allicin alone and in combination with azoles against *Candidas* species. Mycopathologia. 2010;169(4): 287-95.
20. Bakhshi M, Taheri JB, Shabestari SB, Tanik A, Pahlevan R. Comparison of therapeutic effect of aqueous extract of garlic and nystatin mouthwash in denture stomatitis. Gerodontology. 2012; 29(2):e680-4.
21. Gruhlke MC, Portz D, Stitz M, Anwar A, Schneider T, Jacob C, Schlaich NL, Slusarenko AJ. Allicin disrupts the cell's electrochemical potential and induces apoptosis in yeast. Free Radic Biol Med. 2010;49(12):1916-24.
22. An M, Shen H, Cao Y, Zhang J, Cai Y, Wang R, Jiang Y. Allicin enhances the oxidative damage effect of amphotericin B against *Candida albicans*. Int J Antimicrob Agents. 2009;33(3):258-63.
23. Goncagul G, Ayaz E. Antimicrobial effect of garlic (*Allium sativum*). Recent Pat Antiinfect Drug Discov. 2010;5(1):91-3.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20694