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Article

Antifungal Activity of the Essential Oil of *Cymbopogon citratus* (DC) Stapf. An *in vitro* study.

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Abstract: Aim. To evaluate the antifungal potential of the essential oil of *Cymbopogon citratus* by determining the Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) for *Candida albicans* (ATCC 90029), *Candida albicans* (CBS 562), *Candida tropicalis* (ATCC 705) and *Candida tropicalis* strains (CBS 94), as well as to analyze the possible mechanism of action of the oil through the addition of sorbitol to the culture medium. Methods. For the MIC determination, inocula were previously adjusted through spectrophotometry and 100µL were added to the wells of plates already containing the culture medium and 100µL of the serial dilutions of the oil, incubating them in aerobiosis for 24 hours, with subsequent staining by 1% TCT. For the MFC, 50µL of the supernatant from the MIC assay wells were dripped onto Petri dishes and incubated in aerobiosis for 24 hours. Tests were performed in triplicate and data analysed by descriptive statistics. Results. It was determined that the MIC for *Candida albicans* was 125 µg/mL while MIC for *Candida tropicalis* was 250 µg/mL, with the essential oil presenting fungicidal effect for both analyzed yeasts. Conclusion. The essential oil of *Cymbopogon citratus* does not act at the cellular wall level and demonstrated an antimicrobial effect on *Candida albicans* and *Candida tropicalis,* therefore acting as a fungicide.

Keywords: Phytotherapy; microbial sensitivity test; candidiasis.

INTRODUCTION.

A biofilm is a complex three-dimensional structure generally adhered to a solid surface, known as substrate, with time representing a favorable factor for biofilm maturation, resulting in the adsorption of a greater number of microbial species and in a greater degree of interspecies interaction, thus making it difficult to remove or disorganize.[1,2]

In medicine, fungi are responsible for infections, mainly of the skin, gastrointestinal tract and respiratory system, and the biofilm structure is responsible for antimicrobial resistance and for the difficulty in treating these diseases.[1] Yeasts of the genus *Candida spp*. are strongly resistant to antifungal therapies, since they can organize themselves into biofilms,[3] causing serious infections with high morbidity and mortality rates, even in the hospital environment.[4-6] In dentistry, *Candida albicans* biofilms, besides causing diseases such as candidiasis and other opportunistic infections, can cause changes in the mechanical properties of glass ionomer cement, such as reduction of surface hardness after exposure to the biofilm formed by these microorganisms.[7]

Regular and adequate biofilm control is the best way to prevent diseases,

and the use of auxiliary substances with antimicrobial effect[2,4] is recommended. Plant products may present antimicrobial activity and contribute to the chemical control of the biofilm.[3] This makes the possibility of innovation of therapeutic proposals more effective with less potential to promote undesirable effects,[8-10] as in the case of *Cymbopogon citratus,* known by several popular names: *capim-santo, capim-limão, capim-cidreira,* among others.[11-14] Antimicrobial, anti-inflammatory, antiproliferative of tumor-cells and wound-healing properties make *Cymbopogon citratus* a potentially beneficial product for use in the health care area.[15-17] In this context, the aim of this study was to evaluate the antifungal effect of the essential oil of *Cymbopogon citratus,* as well as to verify the possible mechanism of action of the vegetal active pharmaceutical ingredient under testing.

MATERIALS AND METHODS.

Chemical Characterization

The essential oil of *Cymbopogon citratus* was obtained from a reference company in the marketing of essential oils and essences (Quinari Fragrâncias e Cosméticos Ltda., Ponta Grossa, PR, Brazil). It was chemically analyzed in order to compare the results to the information in the leaflet provided by the producer. The essential oil was prepared for Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) evaluations.

Minimum Inhibitory Concentration Determination

Standard *Candida albicans* ATCC 90029, *Candida albicans* CBS 562, *Candida tropicalis* ATCC 705 and *Candida tropicalis* CBS 94 strains were reactivated in Sabouraud agar medium (BD, Heidelberg, Germany) and inocula were prepared the day before the test in aerobiosis and adjusted by spectrophotometry to $5x10^6$ CFU/ml (at 530nm), corresponding to 0.5 in the McFarland scale. Then, the inoculum was diluted, and the final concentration in the plate wells was $2.5x10^3$ CFU/ml.

For the MIC determination, the Serial Microdilution technique methodology was used,[18] and 100µL of Sabouraud broth medium (BD, Heidelberg, Germany) was added to the wells of plates, into which 100µL of the essential oil were added and serially transferred from well to well to achieve the test dilutions, ranging from 2000µg/mL to 15.6µg/mL. Finally, 100µL of the previously adjusted inocula were added. Assays were run in triplicate and the plates incubated in aerobiosis at 37ºC for 24 hours.

Controls of the viability of the test strains were carried out, using medium with inoculum only, and positive control, using medium with inoculum and nystatin solution starting at 100µg/mL. After the 24 hour period, 50µl of 1% TCT solution (2,4,6-Trichloro-1,3,5-triazine) was placed in the wells of plates, to colorimetrically determine the MIC, by confirming the viability of the microorganisms as reflected by the activity of dehydrogenase enzymes involved in the process of the cellular respiration,[16] and which were then incubated for a further 24 hours at 37ºC.

Minimum Fungicide Concentration Determination

To determine the Minimum Fungicide Concentration, 50µL from the wells corresponding to MIC, MICx2 and MICx4 were dripped onto Petri dishes containing Sabouraud Dextrose agar medium (BD - Germany), and incubated in aerobiosis for 24 hours at 37ºC.[17]

Mechanism of Action: Analysis with Addition of Sorbitol

To determine the mechanism of antifungal action against *Candida albicans* ATCC 90029, Sorbitol, a component present in the cell wall, was added to the test culture medium at a concentration of 0.8 M, acting as an osmotic protector.[8]

The test followed the standard protocol for MIC determination using antifungal Caspofugin at concentration 2.5µg/ml as a positive control, since this drug has a mechanism of action known to inhibit fungal cell wall synthesis.

The MIC values with the addition of Sorbitol were determined. Considering that the test product, by inhibiting cell wall synthesis, will stimulate the yeast to use the Sorbitol richly available in the culture medium, it will lead to increased fungal growth.[20] Therefore, in the presence of Sorbitol the MIC should be observed at higher values than those presented for MIC determined in plates containing culture medium only.[19]

Chemical Characterization

The test substance was characterized by chromatography using gas chromatograph coupled to mass spectrometer (GCMS Shimadzu model QP2010, Japan).

Statistical analysis

The results were tabulated and analyzed using descriptive statistics.

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Table 1. MIC and MFC of *Cymbopogon citratus* and positive control on *Candida spp.*

STRAIN	Cymbopogon citratus		Nystatin	
	MIC µg/ml	MFC µg/ml	MIC µg/ml	MFC µg/ml
Candida albicans ATCC 90029	125	125	0.4	0.8
Candida albicans CBS 562	125	125	0.4	0.8
Candida tropicalis ATCC 705	250	250	0.4	0.4
Candida tropicalis CBS 94	250	250	0.4	0.4

Table 2. MIC obtained with and without the osmotic protector Sorbitol, against *Candida albicans.*

STRAIN	*C. citratus*		Caspofungin	
	MIC µg/ml	MIC Sorbitol µg/ml	MIC µg/ml	MIC Sorbitol µg/ml
Candida albicans ATCC 90029	125	125	0.078	0.15

RESULTS.

The chemical characterization showed that the major component of the product is citral, corresponding to approximately 84% of its constituents, a little more than the 80% informed in the leaflet from the producer. Other constituents were identified as myrcene and geraniol, but at very low concentrations.

The data obtained in the antimicrobial evaluation allow defining the vegetal active principle as antifungal, with fungicidal effect, without alterations by the addition of Sorbitol to the culture medium (Table 1 and Table 2). The fungicidal effect was observed on the MFC test, which did not detect fungal growth.

DISCUSSION.

The use of phytotherapeutic agents in dentistry has been an alternative therapeutic option[21] and the most important group of active compounds in dental formulations of natural origin include essential oils and monoterpenoids.[22]

It is suggested that terpenes are phyto-constituents responsible for the antimicrobial activity of this essential oil, with the main focus on citral,[23,24] which is the major component of the test product, and which may be responsible for the antifungal effect observed. However, differences in constituents may occur according to variations on the location and time of collection of the botanical samples of *Cymbopogon citratus*.[25] Such variations make it necessary to verify the composition of plant active principles used in studies, justifying the CG/MS analysis applied here, in order to be able to make comparisons with other studies.

The antifungal effect observed in this study corro-borates the literature findings, showing action on *Candida spp.* species at low concentrations.[7,15,26] However, there is disagreement about the fungicide potential, since some authors observed a fungistatic effect.[15] A previous study has shown that even though antifungal activity on clinical *Candida albicans* and *Candida tropicalis* isolates was found, the essential oil of *Cymbopogon citratus* was effective only on 70% of *Candida albicans* isolates and 50% of *Candida tropicalis* isolates.[15]

When using the agar diffusion methodology with the essential oil in the liquid and vapor phase, some authors reported a potent inhibitory effect of the oil,[16] and the results obtained for the vapor phase test were superior to those of the liquid phase, suggesting that this finding may be due to volatile compounds present in the oil. However, another study demonstrated that cellular defects were more evident for the vapor phase.[26]

The results of the present study for the MIC of *Candida albicans* (125µg/mL) are lower than those described by other authors, who found MIC of 288µg/mL.[26] This result can be justified by climate variations and the manner in

which the material used to produce the oil was collected, resulting in the vegetal active principle being used in this research having a higher amount of citral.

There is no consensus in the literature regarding the concentration of the substance that can determine its microbicidal potential, and it may be possible to attribute different classifications to the essential oil studied here. The results obtained for MIC in this study can be classified as very good by some authors, since they are below 1mg/mL.[27]

However, for other researchers, the classification would be of potent substance, since the MIC was lower than 500µg/mL.[31] Finally, differing from previous studies,[27,28] the effect of the essential oil on yeast in this study can be considered moderate, as the MIC is between 100 and 500µg/mL.[29]

Reinforcing the importance of studies using the essential oil of *Cymbopogon citratus* as study material, the literature also highlights this vegetal active principle as promising to prepare mouthwashes, dental creams and to be part of the therapeutic arsenal used in Dentistry. Considering that many biofilm infections due to *Candida spp*. may begin in the oral cavity[3] the control of the dental biofilm through the use of this substance may be a prophylactic alternative.[30]

The literature also reports the anti-inflammatory potential as an advantage of its topical use, which at concentrations of 10mg/kg or 10µL/area was able to reduce atrial edema in animal models.[17] These findings instigate new investigations and place the essential oil of *Cymbopogon citratus* in a favorable condition to be applied in mouthwash solutions, which is the proposal of this research.

Antifungal Caspofungin acetate inhibits the synthesis of essential constituents of the cell wall[31] and was chosen as a positive control in the present research. When compared to findings for the essential oil, the fungal strains studied did not respond to the addition of Sorbitol to the culture medium when treated with *Cymbopogon citratus,* indicating that the cell wall is not the site of action of the essential oil in these microorganisms and instigates the performance of further studies in order to elucidate the still not clarified mechanism of action of this vegetal material.

Since essential oils have many associated phytocomponents that can act synergistically at various sites, it is difficult to identify a specific and unique mechanism of action. However, when the mechanism of antimicrobial action is due to interference with the cell wall of the microorganism, there is an advantage for its medicinal use because of cell selectivity, being less toxic to human cells.[19]

CONCLUSION.

The essential oil of *Cymbopogon citratus* does not act at the cellular wall level and has demonstrated antimicrobial effects on *Candida albicans* and *Candida tropicalis*, being therefore a fungicide.

REFERENCES.

1. Sandai D, Tabana YM, Ouweini AE, Ayodeji IO. Resistance of Candida albicans Biofilms to Drugs and the Host Immune System. Jundishapur J Microbiol. 2016;9(11):e37385.
2. Yu SB, Li WG, Liu XS, Che J, Lu JX, Wu Y. The Activities of Adhesion and Biofilm Formation by Candida tropicalis Clinical Isolates Display Significant Correlation with Its Multilocus Sequence Typing. Mycopathologia. 2017;182(5-6):459–69.
3. de Castro R, Lima EO. Screening da Atividade Antifúngica de Óleos Essenciais sobre Cepas de Candida. Pesq Bras Odontoped Clin Integr. 2011;11(3):341–5.
4. Ghannoum M, Roilides E, Katragkou A, Petraitis V, Walsh TJ. The Role of Echinocandins in Candida Biofilm-Related Vascular Catheter Infections: In Vitro and In Vivo Model Systems. Clin Infect Dis. 2015;61(Suppl 6):S618–21.
5. Hassaine-Lahfa I, Boucherit-Otmani Z, Sari-Belkherroubi L, Boucherit K. Retrospective study of Candida sp. contaminations of endoscopes at the University Hospital of Tlemcen (Algeria) J Mycol Med. 2017;27(2):127–32.
6. Goel S, Mittal S, Chaudhary U. Role of Non Albicans Candida Spp. and Biofilm in Neonatal ICU. Infect Disord Drug Targets. 2016;16(3):192–8.
7. Bertolini MM, de Oliveira G, Charone S, Soares MA, de Souza IPR, Portela MB. Avaliação in vitro da Microdureza de Cimentos de Ionômero de Vidro Modifi cados por Resina Submetidos a Biofi lme de Candida albicans. Pesq Bras Odontoped Clin Integr. 2010;10(2):249–55.
8. Peixoto LR, Rosalen PL, Ferreira GL, Freires IA, de Carvalho FG, Castellano LR, de Castro RD. Antifungal activity, mode of action and anti-biofilm effects of Laurus nobilis Linnaeus essential oil against Candida spp. Arch Oral Biol. 2017;73:179–85.
9. de Castro RD, de Souza TM, Bezerra LM, Ferreira GL, Costa EM, Cavalcanti AL. Antifungal activity and mode of action of thymol and its synergism with nystatin against Candida species involved with infections in the oral cavity: an in vitro study. BMC Complement Altern Med. 2015;15:417.
10. Pereira JV, Freires IA, Castilho AR, da Cunha MG, Alves Hda S, Rosalen PL. Antifungal potential of Sideroxylon obtusifolium and Syzygium cumini and their mode of action against Candida

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albicans. Pharm Biol. 2016;54(10):2312–9.
11. Liporacci HSN, Simão DG. Levantamento etnobotânico de plantas medicinais nos quintais do Bairro Novo Horizonte, Ituiutaba, MG. Rev Bras Plantas Med. 2013;15(4):529–40.
12. de Oliveira GS, de Oliveira AFM, Andrade LHC. Plantas medicinais utilizadas na comunidade urbana de Muribeca, Nordeste do Brasil. Acta Bot Bras. 2010;24(2):571–7.
13. Vásquez SPF, de Mendonça MS, Noda SN. Etnobotânica de plantas medicinais em comunidades ribeirinhas do Município de Manacapuru, Amazonas, Brasil. Acta Amaz. 2014;44(4):457–72.
14. Zucchi MR, Oliveira Júnior VF, Gussoni MA, Silva MB, Silva FC, Marques NE. Levantamento etnobotânico de plantas medicinais na cidade de Ipameri - GO. Rev Bras Plantas Med. 2013;15(2):273–9.
15. Almeida RBA, Akisue G, Cardoso LML, Junqueira JC, Jorge AO. Antimicrobial activity of the essential oil of Cymbopogon citratus (DC) Stapf. on Staphylococcus spp., Streptococcus mutans and Candida spp. Rev Bras Plantas Med. 2013;15(4):474–82.
16. Deswal DP, Chand U. Standardization of the tetrazolium test for viability estimation in ricebean (Vigna umbellata (Thunb.) Ohwi e Ohashi) seeds. Seed Sci Tech. 1997;25(3):409–17.
17. Boukhatem MN, Ferhat MA, Kameli A, Saidi F, Kebir HT. Lemon grass (Cymbopogon citratus) essential oil as a potent anti-inflammatory and antifungal drugs. Libyan J Med. 2014;9(1):25431.
18. Halabi MF, Sheikh BY. Anti-proliferative effect and phytochemical analysis of Cymbopogon citratus extract. Biomed Res Int. 2014;2014:906239.
19. Lira de Souza Sales Rocha EA, Dantas de Medeiros AC, Dias de Castro R, Rosalen PL, Alcântara Saraiva KL, Pina Godoy G, Rodrigues Apolinário da Silva L, Sousa Silva Aleixo C, Guimarães Silva P, Melo de Brito Costa EM. Antifungal Activity, Phytochemical Characterization and Thermal Profile of Anadenanthera colubrina (Vell.) Brenan. Pesq Bras Odontoped Clín Integr. 2017;17(1):1–14.
20. Guedes da Silva I, Bandeira de Pontes Santos H, Wanderley Cavalcanti Y, Weege Nonaka C, Alves de Sousa S, Dias de Castro R. Antifungal Activity of Eugenol and its Association with Nystatin on Candida albicans. Pesq Bras Odontoped Clín Integr. 2017;17(1):1–8.
21. Nóbrega DRM, Santos RL, Soares RSC, Alves PM, Medeiros ACD, Pereira JV. A randomized, controlled clinical trial on the clinical and microbiological efficacy of Punica granatum Linn mouthwash. Pesq Bras Odontoped Clin Integr. 2015;15(1):301–8.
22. Nogueira MNM, Correia MF, Fontana A, Bedran TBL, Spolidorio DMP. In vivo comparative evaluation of the efficacy of Melaleuca oil, chlorhexidine and listerine against streptococcus mutans and total microorganisms in saliva. Pesq Bras Odontoped Clin Integr. 2013;4(13):343–9.
23. Lucena BFF, Tintino SR, Figueredo FG, Oliveira CDM, Aguiar JJS, Cardoso EN, Aquino PEA, Andrade JC, Coutinho HDM, Matias EFF. Avaliação da atividade antibacteriana e moduladora de aminoglicosídeos do óleo essencial de Cymbopogon citratus (DC.) Stapf. Acta biol Colomb. 2015;20(1):39–45.
24. Leite MC, Bezerra AP, de Sousa JP, Guerra FQ, Lima Ede O. Evaluation of Antifungal Activity and Mechanism of Action of Citral against Candida albicans. Evid Based Complement Alternat Med. 2014;2014:378280.
25. Furlan MR, Martins RCC, Rodrigues E, Scalco N, Negri G, Lago JHG. Variação dos teores de constituintes voláteis de Cymbopogon citratus (DC) Staf, Poaceae, coletados em diferentes regiões do Estado de São Paulo. Rev Bras farmacogn. 2017;20(5):686–91.
26. Tyagi AK, Malik A. Liquid and vapour-phase antifungal activities of selected essential oils against Candida albicans: microscopic observations and chemical characterization of Cymbopogon citratus. BMC Complement Altern Med. 2010;10:65.
27. Aligiannis N, Kalpoutzakis E, Mitaku S, Chinou IB. Composition and antimicrobial activity of the essential oils of two Origanum species. J Agric Food Chem. 2001;49(9):4168–70.
28. Duarte MC, Leme EE, Delarmelina C, Soares AA, Figueira GM, Sartoratto A. Activity of essential oils from Brazilian medicinal plants on Escherichia coli. J Ethnopharmacol. 2007;111(2):197–201.
29. Holetz FB, Pessini GL, Sanches NR, Cortez DA, Nakamura CV, Filho BP. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz. 2002;97(7):1027–31.
30. Rajesvari R, Lakshmi T. Lemon grass oil for improvement of oral health. Dent Hypotheses. 2013;4(4):115–7.
31. Song JC, Stevens DA. Caspofungin: Pharmacodynamics, pharmacokinetics, clinical uses and treatment outcomes. Crit Rev Microbiol. 2016;42(5):813–46.