ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1067-1070.
https://doi.org/10.3889/oamjms.2019.245
eISSN: 1857-9655
Basic Science

The Effects of Cinnamaldehyde (Cinnamon Derivatives) and Nystatin on *Candida Albicans* and *Candida Glabrata*

Sedigheh Bakhtiari[1], Soudeh Jafari[1], Jamileh Bigom Taheri[1], Tahereh Sadat Jafarzadeh Kashi[2], Zahra Namazi[2*], Maryam Iman[3], Mohammad Poorberafeyi[1]

[1]Department of Oral Medicine, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Department of Dental Biomaterial, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran; [3]Chemical Injuries Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

Citation: Bakhtiari S, Jafari S, Taheri JB, Kashi TSJ, Namazi Z, Iman M, Poorberafeyi M. The Effects of Cinnamaldehyde (Cinnamon Derivatives) and Nystatin on *Candida Albicans* and *Candida Glabrata*. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1067-1070. https://doi.org/10.3889/oamjms.2019.245

Keywords: Cinnamon; Cinnamaldehyde; Nystatin; Candida albicans; Candida glabrata

***Correspondence:** Zahra Namazi. Department of Dental Biomaterials, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran. E-mail: zahra.t.namazi@gmail.com

Received: 19-Feb-2019; **Revised:** 22-Mar-2019; **Accepted:** 23-Mar-2019; **Online first:** 10-Apr-2019

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Candida species are the most common opportunistic fungal infections. Today, cinnamon plants have been considered for anti-Candida properties.

AIM: This study aimed to investigate the effectiveness of cinnamaldehyde extract (from cinnamon derivatives) on *Candida albicans* and *Candida glabrata* species and comparison with nystatin.

MATERIAL AND METHODS: In this study, cinnamaldehyde and nystatin were used. The specimens included *Candida albicans* and *Candida glabrata*. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were measured for each one by the microdilution method. This experiment was repeated three times.

RESULTS: Cinnamaldehyde extract at a concentration of 62.5 µl/ml was able to prevent the growth of *Candida albicans*, at a concentration of 93.7 µl/ml, causing *Candida albicans* to disappear, at 48.8 µl/ml, to prevent the growth of *Candida glabrata*, and in the concentration of 62.5 µl/ml, causes the loss of *Candida glabrata*. In comparison, nystatin at 0.5 µg/ml concentration prevented the growth of *Candida albicans*, at concentrations of 1 µg/ml causing *Candida albicans* to be destroyed, at 4 µg/ml concentration to prevent the growth of *Candida glabrata*, and at a concentration of 8 µg/ml causes the loss of *Candida glabrata*. The results were the same every three times.

CONCLUSIONS: Although cinnamaldehyde extract had an effect on fungal growth in both *Candida albicans* and *Candida glabrata* with a fatal effect; the effect on these two species was lower than nystatin.

Introduction

Candidiasis is an important and common disease of the oral mucosa, caused by various *Candida* species [1], [2]. *Candida albicans* is a major species of pathogens that exist in the form of commensal in the human mouth. The importance of non-albicans such as *Candida glabrata* has increased in recent years [3]. *Candida glabrata* species is known as the most common cause of infection after *Candida albicans* which is seen in about 31-55% of healthy people. These microorganisms can become pathogens with weakening the immune system [4], [5].

Cinnamon zeylanicum blume has been used as an aromatic herb for a long time in the Asian and European herbal medicine for food and medicine application. This herb has been used in traditional medicine for treatment of respiratory, digestive and genital tract defects [6]. The majority of cinnamon essential oil is cinnamaldehyde [7]. Various studies have been done on cinnamon properties [8], [9], [10]. They considered cinnamon as an antioxidant, anti-inflammatory, anti-bacterial, anti-diabetes, anticancer and lipid-lowering agent. The standard topical drug to resolve *candida* infection is nystatin mouthwash. However the inappropriate taste of nystatin mouthwash and repeated preparation and use of it during the day leads to dissatisfaction of the patients. Hence the presence of mouthwashes with proper flavour, easier use, and proper effect seems necessary [11].

Few reports showed the positive effects of cinnamon on the treatment of *Candida albicans* [8], [9], [10] and *Candida glabrata* [9], and limited studies have investigated its comparative effects with nystatin therapy [8], [12]. Providing such herbal medicines that have antimicrobial effects can be useful in reducing side effects and toxic effects on the tissue, as well as economically viable [13], [14], [15].

The purpose of the present study was to evaluate the effects of Cinnamaldehyde (cinnamon derivatives) and Nystatin on *Candida albicans* and *Candida glabrata*. Furthermore, we compared the antifungal effects of Cinnamaldehyde and Nystatin relative to each other using an experimental laboratory intervention method.

Material and Methods

An experimental laboratory intervention was designed at the Institute of Biology and Poisoning, School of Medicine, Baqiyatallah University of Medical Sciences. The studied samples included standard species (ATCC*) Candida albicans* (ATCC 90028) and *Candida glabrata*, both of which were obtained from the Mycology Laboratory of the School of Veterinary Medicine, University of Tehran. The present study was carried out using pure cinnamaldehyde (purchased from Merck Germany) and nystatin (purchased from Sigma).

To determine the lowest inhibitory concentration of cinnamaldehyde extract on two strains *Candida albicans* and *Candida glabrata*, we used eight different concentrations. For each microorganism, a plate was used to evaluate the effect of cinnamaldehyde extract, and overall two plates for two types of *candida* were considered. To prepare the required dilutions for nystatin, according to the protocol of the Sigma factory, 1.024 mg of nystatin powder were added to 1 ml of 5% Dimethyl sulfoxide (DMSO) and a concentration of 1.024 mg/ml of nystatin obtained. The culture media were prepared according to the manufacturer's instructions. Then, the *candidates* prepared on the culture medium with sterile inoculating loops and the plate was incubated for 24 hours in a 37°C incubator, and then candida colonies appeared on the culture medium. Fungal suspensions were prepared according to the turbidity McFarland criterion 0.5. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of cinnamaldehyde extract were determined by dilution in the tube. To determine the MIC for a series of eight tubes, we tested the dilutions of each extract. Also, a tube as a positive control containing diluted extract and a negative control medium containing microbial suspension and culture medium was considered. A total of eight dilution series of cinnamaldehyde extract was prepared as 187.5, 125, 93.75, 62.5, 46.8, 31.2, 23.4, 15.6 µl/ml. According to the microdilution method contained in CLSI, in each of the eight microplates, these concentrations, plus 10 µl of fungal suspension were placed. They were incubated 24 hours at 37°C and eventually the first transparent well that indicated the lack of microorganism growth was considered as MIC. The contents of wells without growth were cultured in a solid culture medium, and after 24 hours of incubation, the lowest concentration in which growth was not observed was considered as MFC. To control the test, positive control and negative control were considered, so that we used the culture medium and *candida* for positive control and the culture medium and cinnamaldehyde for negative control. Ethics committee approval was not required for this study.

Regarding the fact that in this type of microbiological experiment, the trials are repeated three times, in the present study, for each *candida* in each concentration, three replications were repeated. The results of the study were reported descriptively in different groups of intervention and control.

Results

To detect the antifungal effects of cinnamaldehyde extract and nystatin on the growth of two species of *Candida albicans* and *Candida glabrata*, we used the microdilution method to determine MIC and MFC with three replications.

Table 1: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Cinnamaldehyde Extract on Candida albicans and Candida glabrata growth

Row	Concentrations of cinnamaldehyde extract(µg/ml)	Candida albicans		Candida glabrata	
		MIC	MFC	MIC	MFC
1	187/5	-	-	-	-
2	125	-	-	-	-
3	93/75	+	-	-	-
4	62/5	+	+	+	-
5	46/8	+	+	+	+
6	31/2	+	+	+	+
7	23/4	+	+	+	+
8	15/6	+	+	+	+

This method was also used to determine the MIC and MFC of nystatin. The results of MIC and MFC for cinnamaldehyde and nystatin are presented in Table 1, 2, and 3.

Table 2: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Nystatin on Candida albicans

	Sample (oil)		Nystatin	
Fungi	MIC (µl/ml)	MFC (µl/ml	MIC (µg/ml)	MFC (µg/ml)
Candida albicans ATCC 90028	62.5	93.7	0.5	1

This experiment was repeated three times. We also compared the MIC and MFC of cinnamaldehyde and nystatin on the growth of

Candida albicans and *Candida glabrata* (Figure 1).

Table 3: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Nystatin on Candida glabrata

Fungi	Sample (oil)		Nystatin	
	MIC (µl/ml)	MFC(µl/ml)	MIC (µg/ml)	MFC (µg/ml)
Candida glabrata ATCC	48.8	62.5	4	8

The results indicated that nystatin, with a much lower concentration than cinnamaldehyde extract, exerts a lethal effect on *Candida albicans* and *Candida glabrata*.

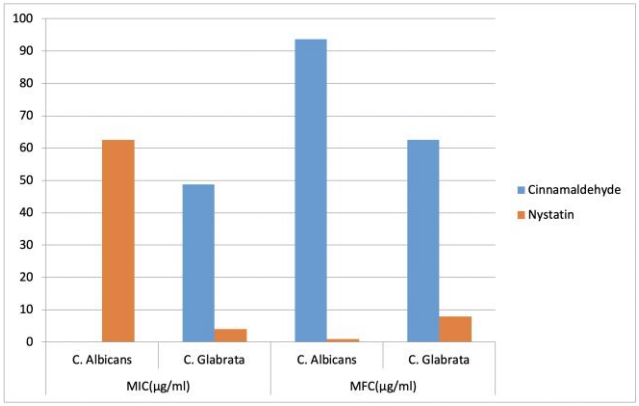

Figure 1: Comparison of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Cinnamaldehyde extract and Nystatin on Candida albicans and Candida glabrata

Discussion

Topical treatment for oral candidiasis is nystatin mouthwash, which its requirement to frequent use and unpleasant taste, often leads to patients' dissatisfaction and sometimes lack of continuity of treatment. For this reason, offering a proper flavour mouthwash with the ease of use and proper efficacy seems necessary and practical [16]. Studies conducted in the last decade clearly show the unpleasant effects of chemical drugs alongside their beneficial effects. Recent studies have paid more attention to the use of medicinal plants due to increased drug resistance to new chemical drugs. This study aimed to evaluate the antifungal effects of cinnamaldehyde extract against *Candida albicans* and *Candida glabrata*, and compare it with the antifungal effect of nystatin. Most previous studies have been conducted on specific pathogens that affect the skin, respiratory, digestive and urogenital system, and few studies have been done on oral mucosal pathogens [17], [18], [19]. For many years, *Candida glabrata* was considered a relatively non-pathogenic saprophytic flora of normal people, unconnected with a serious infection in humans. However, following the large and widespread use of immunosuppressive agents, along with treatment with broad-spectrum antibiotics, the number of systemic and mucosal infections caused by *Candida glabrata* has increased dramatically [20]. In Fani and Kohanteb (2011) [9], the antifungal effects of cinnamon on *Candida albicans* are mentioned. Also, Casrto and Lima (2013) pointed to the antifungal effects of essential oil of cinnamon, which the main component of it is eugenol, on *Candida albicans*, *Candida tropicalis*, and *Candida krusei*. They found the direct effect of cinnamon essential oil on the cell wall synthesis of the yeast [21]. Many researchers have indicated that there is a correlation between the chemical composition and the antifungal activity. They also showed that the strong antifungal activity of the oils derived from the skin and the cinnamon leaf was due to the high levels of cinnamaldehyde (44.2%) and eugenol (90.2%) [22]. However, other compounds may also contribute to antifungal activity. The study of Arbabi et al., (2011) aimed at comparing the effect of thyme, cloves and cinnamon extract with nystatin on the inhibition of *Candida albicans*. The diameter of the inhibition zone of each plant extract was compared with the diameter of the positive control growth zone with ANOVA. Candida inhibition in the nystatin group was 32.6 ± 0.84, and in cinnamon, extract group was 31.3 ± 0.82. In addition, *candida* inhibition in clove extract group was 27.4 ± 0.82 and finally in thyme evaluated 13 ± 0.82. As a result, the highest effect was first on nystatin, cinnamon, cloves and ultimately thyme ($P < 0.000$). The final result was that thyme, cloves, and cinnamon had antifungal effects on the *Candida albicans* [12]. However, in these two studies, the method of measuring the inhibitory effect of cinnamon extract is not the same, but both studies have confirmed that cinnamon has antifungal effects. The aim of the study of Ataei et al., (2007) was an experimental evaluation on the antifungal effects of absinthium Artemisia, eucalyptus, onion, cinnamon, turmeric, sage, mint, and Calendula officinalis on a standard strain of *Candida albicans* compared to nystatin mouthwash. The results showed that the extract of each of the six herbs had an antifungal effect. Cinnamon has been shown to be more potent and more effective than the onion, mint, Calendula officinalis and sage, with the same effect as turmeric, absinthium Artemisia and eucalyptus. Also, cinnamon, absinthium Artemisia, eucalyptus exhibited significant antifungal effects in comparison with nystatin [8]. Carvalhinho et al., (2012) and Condò et al., (2018) also showed a significant activity of cinnamon against *Candida albicans* [23], [24]. Gucwa et al., (2018) exhibited both fungistatic and fungicidal activity of some plant oils toward *Candida albicans* and *Candida glabrata* isolates and the highest activity was demonstrated for cinnamon oil [25]. The results of these studies confirm the result of the present study and the antifungal properties of cinnamon.

In conclusion, although the cinnamaldehyde extract in comparison with nystatin can prevent the

growth and loss of *Candida albicans* and *Candida glabrata* with higher concentrations; given the antifungal effects of this plant, it is expected that it could be considered as an effective medicinal plant. Since limited studies have been conducted on cinnamon plants, it is suggested to perform extraction of other active substances and its compounds, molecular studies to understand the mechanisms of action of the ingredients, Invivo tests in the animal model following Invitro tests and cell culture experiments.

Acknowledgements

We thank our colleagues from Shahid Beheshti University of Medical Sciences and Baqiyatallah University of Medical Sciences who provided insight and expertise that greatly assisted the research, although they may not agree with all of the conclusions of this paper.

References

1. Lalla RV, Patton LL, Dongari-Bagtzoglou A. Oral candidiasis: pathogenesis, clinical presentation, diagnosis and treatment strategies. Journal of the California Dental Association. 2013; 41(4):263-8. PMid:23705242

2. Millsop JW, Fazel N. Oral candidiasis. Clinics in dermatology. 2016; 34(4):487-94. https://doi.org/10.1016/j.clindermatol.2016.02.022 PMid:27343964

3. Mortazavi H, Safi Y, Baharvand M, Jafari S, Anbari F, Rahmani S. Oral White Lesions: An Updated Clinical Diagnostic Decision Tree. Dentistry journal. 2019; 7(1):15. https://doi.org/10.3390/dj7010015 PMid:30736423

4. Suvirya S, Gandhi R, Agarwal J, Patil R. Erythematous candidiasis leading to systemic manifestations of human immunodeficiency virus co-infection with secondary syphilis: a diagnostic and therapeutic dilemma. European journal of dentistry. 2015; 9(3):449. https://doi.org/10.4103/1305-7456.163219 PMid:26430379 PMCid:PMC4570002

5. Gürgan CA, Zaim E, Bakirsoy I, Soykan E. Short-term side effects of 0.2% alcohol-free chlorhexidine mouthrinse used as an adjunct to non-surgical periodontal treatment: a double-blind clinical study. Journal of periodontology. 2006; 77(3):370-84. https://doi.org/10.1902/jop.2006.050141 PMid:16512751

6. Gruenwald J, Freder J, Armbruester N. Cinnamon and health. Critical reviews in food science and nutrition. 2010; 50(9):822-34. https://doi.org/10.1080/10408390902773052 PMid:20924865

7. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evidence-Based Complementary and Alternative Medicine. 2014; 2014:642942.

8. Ataei Z, Ansari M, AYAT EM, Mirzaei A. In-vitro study of antifungal effects of selected herbal extracts on standard and wild strains of Candida albicans. The Journal of Islamic Dental Association of IRAN. 2007; 19:91-97

9. Fani MM, Kohanteb J. Inhibitory activity of Cinnamomum zeylanicum and eucalyptus globulus oils on Streptococcus mutans, Staphylococcus aureus, and Candida species isolated from patients with oral infections. Journal of Dentistry, Shiraz University of Medical Sciences. 2011; 11:14-22.

10. Almeida LD, Paula JF, Almeida RV, Williams DW, Hebling J, Cavalcanti YW. Efficacy of citronella and cinnamon essential oils on Candida albicans biofilms. Acta Odontologica Scandinavica. 2016; 74(5):393-8. https://doi.org/10.3109/00016357.2016.1166261 PMid:27098375

11. Lyu X, Zhao C, Yan ZM, Hua H. Efficacy of nystatin for the treatment of oral candidiasis: a systematic review and meta-analysis. Drug design, development and therapy. 2016; 10:1161–1171. https://doi.org/10.2147/DDDT.S100795 PMid:27042008 PMCid:PMC4801147

12. SHerzaee M, Poorzamani M. Inhibitory Effects of Plant Extracts Containing Thyme, Clore and Cinnamon Compared to Nystatin On Candida Albicans.(Invitro). Res Dent Sci. 2012; 8(4):175-9.

13. Baharvand M, Jafari S, Mortazavi H. Herbs in oral mucositis. Journal of clinical and diagnostic research. 2017; 11(3):ZE05–ZE11. https://doi.org/10.7860/JCDR/2017/21703.9467

14. Shekar BR, Nagarajappa R, Suma S, Thakur R. Herbal extracts in oral health care-A review of the current scenario and its future needs. Pharmacognosy reviews. 2015; 9(18):87–92. https://doi.org/10.4103/0973-7847.162101 PMid:26392704 PMCid:PMC4557240

15. Li CL, Huang HL, Wang WC, Hua H. Efficacy and safety of topical herbal medicine treatment on recurrent aphthous stomatitis: a systemic review. Drug design, development and therapy. 2016; 10:107–115. PMid:26770058

16. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of ethnopharmacology. 2001; 74(2):113-23. https://doi.org/10.1016/S0378-8741(00)00335-4

17. Herrera-Arellano A, López-Villegas EO, Rodríguez-Tovar AV, Zamilpa A, Jiménez-Ferrer E, Tortoriello J, Martínez-Rivera MA. Use of antifungal saponin SC-2 of Solanum chrysotrichum for the treatment of vulvovaginal candidiasis: in vitro studies and clinical experiences. African Journal of Traditional, Complementary and Alternative Medicines. 2013; 10(3):410-7. https://doi.org/10.4314/ajtcam.v10i3.4

18. Taguchi Y, Ishibashi H, Takizawa T, Inoue S, Yamaguchi H, Abe S. Protection of oral or intestinal candidiasis in mice by oral or intragastric administration of herbal food, clove (Syzygium aromaticum). Nippon Ishinkin Gakkai Zasshi. 2005; 46(1):27-33. https://doi.org/10.3314/jjmm.46.27

19. Sheidaei S, Sadeghi T, Jafarnejad F, Rajabi O, Najafzadeh M. Herbal medicine and vaginal candidiasis in Iran: a review. Evidence Based Care. 2017; 7(2):71-7.

20. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS microbiology reviews. 2012 Mar 1; 36(2):288-305. https://doi.org/10.1111/j.1574-6976.2011.00278.x PMid:21569057

21. Castro RD, Lima EO. Anti-candida activity and chemical composition of Cinnamomum zeylanicum blume essential oil. Brazilian Archives of Biology and Technology. 2013; 56(5):749-55. https://doi.org/10.1590/S1516-89132013000500005

22. Tabassum N, Vidyasagar G. Antifungal investigations on plant essential oils. A review . Int J Pharm Sci. 2013; 5:19-28

23. Carvalhinho S, Costa AM, Coelho AC, Martins E, Sampaio A. Susceptibilities of Candida albicans mouth isolates to antifungal agents, essentials oils and mouth rinses. Mycopathologia. 2012; 174(1):69-76. https://doi.org/10.1007/s11046-012-9520-4 PMid:22246961

24. Condò C, Anacarso I, Sabia C, Iseppi R, Anfelli I, Forti L, de Niederhäusern S, Bondi M, Messi P. Antimicrobial activity of spices essential oils and its effectiveness on mature biofilms of human pathogens. Natural product research. 2018:1-8. https://doi.org/10.1080/14786419.2018.1490904 PMid:30317865

25. Gucwa K, Milewski S, Dymerski T, Szweda P. Investigation of the antifungal activity and mode of action of Thymus vulgaris, Citrus limonum, Pelargonium graveolens, Cinnamomum cassia, Ocimum basilicum, and Eugenia caryophyllus essential oils. Molecules. 2018; 23(5):1116. https://doi.org/10.3390/molecules23051116 PMid:29738503 PMCid:PMC6099571