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Short Communications

In vitro antifungal effect of cinnamon extract on candida species

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Abstract

Introduction: Cinnamon zeylanicum is used for various medical purposes. The aim of this study was to compare the antifungal efficacy of cinnamon and amphotericin B against Candida species in vitro.

Material & Methods: Candida albicans (C. albicans) and Candida krusei (C. Krusei) were obtained from the Iranian Industrial and Scientific Research Center. The minimum inhibitory concentration (MIC) of cinnamon extract was determined and compared with that of amphotericin B.

Results: The results of this study showed that Amphotericin B, ethanolic and aqueous extract of cinnamon inhibited the growth of C. albicans and C. Krusei with different MICs.

Conclusion: Ethanolic extract of cinnamon has inhibitory effects on Candida species comparable to that of amphotericin B. So, it can be used as a herbal alternative.

Keywords: Amphotericin B, Candida albicans, Cinnamon

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اثر ضد قارچی عصاره دارچین بر روی گونه‌های کاندیدا در محیط آزمایشگاهی

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چکیده

مقدمه: *دارچین* در اهداف مختلف پزشکی مورد استفاده قرار می‌گیرد. هدف از مطالعه حاضر مقایسه اثر بخشی عصاره دارچین و آمفوتریسینB بر روی گونه‌های کاندیدا در محیط آزمایشگاهی می‌باشد.

مواد و روش ها: نمونه های مطالعه شامل گونه‌های*کاندیدا آلبیکنس* و *کاندیدا کروزوئی* بودندکه ازکلکسیون سازمان پژوهش‌های علمی وصنعتی ایران تهیه شد. حداقل غلظت مهار کننده تعیین گردید و اثرات عصاره با آمفوتریسین B بر روی این قارچها مقایسه گردید.

یافته ها: نتایج این مطالعه نشان داد که آمفوتریسین B ، عصاره الکلی و عصاره آبی دارچین با عث مهار رشد کاندیدا آلبیکنس و کاندیدا کروزویی با حداقل غلظت های مختلف شدند..

نتیجه گیری: عصاره الکلی دارچین فعالیت مهارکنندگی چشمگیری برروی مخمرهای کاندیدا نشان می‌دهد. بنابراین می تواند به عنوان جایگزین گیاهی استفاده شود.

واژگان کلیدی: آمفوتریسین B، کاندیدا آلبیکنس، دارچین

Introduction

Assessment of oral microorganisms and their interactions with each other and with the host are among the highly debated topics in oral medicine. As a normal oral microflora, Candida species are present in the mouth of 20-50% of healthy population. C.albicans is the most common cause of oral candidiasis in immunocompromised patients.[1] Amphotericin B is recommended as the first line treatment, and azoles are often prescribed as an alternative for treatment of candidiasis and candidemia.However, it has severe side effects such as nephrotoxicity, significant reduction of glomerular filtration of potassium and rhabdomyolysis. [2] Cinnamomum zeylanicum blume is a reputed spice with a pleasant aroma. In the traditional medicine, it is used for treatment of respiratory, gastrointestinal and genital conditions. It belongs to the Lauraceae family and has antimicrobial, antioxidant, anti-diabetic, antiviral and anti-spastic properties. [3] Some constituents of cinnamon such as cinnamaldehyde, eugenol and limonene have analgesic properties and coumarin, eugenol, cinnamaldehyde and cinnamic acid have anti-inflammatory effects as well. Since pathogenic fungi are eukaryotic, chemical antifungal medications may have adverse effects on host cells as well. [4]

Thus, given that their antifungal efficacy is confirmed, plant extracts may be used as an alternative to chemical antifungals since medicinal plants have fewer side effects and are not hazardous for the environment. This in vitro study sought to assess the effect of cinnamon extract compared to amphotericin B on Candida species.

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Materials &Methods

This in vitro study was conducted on cultured C. albicans and C. krusei species. Collection and identification of cinnamon plant were carried out by a botanist in School of Pharmacy of Shahid Beheshti University of Medical Sciences. Aqueous and ethanolic extracts of cinnamon were tested three times in eight different concentrations and the MIC of extracts compared to that of Amphotericin B was reported. Since the study had a descriptive design, sample size calculation was not required.

Dried cinnamon sticks were milled in an electric milling machine until a homogenous powder was obtained; 200g of this powder was precisely weighed and poured into a beaker; 1000mL of distilled water was also added and the suspension was boiled for 10 minutes. Next, the beaker was capped with aluminum foil for 40 hours and the suspension was then filtered using a paper filter; 20% aqueous extract was then obtained using a bain marie. To obtain ethanolic extract, 200g of cinnamon powder was added to 1000mL of 96% alcohol and the mixture was transferred to a dark container,stored in a cold and dark environment for two days and shaken at regular time intervals during this time period.

The mixture was then filtered using a clean cloth and paper filter and the obtained solution was centrifuged at 4000 rpm for 10 minutes. The supernatant was transferred to a glass dish and stored for 10 days to dry; 20% ethanolic extract was obtained as such.[5] Standard strains (Persian Type Culture Collection) of C. albicans (PTCC:5297) and C. krusei (PTCC:5027) were obtained from the Scientific and Industrial Research Organization. Amphotericin B was selected as the gold standard for the purpose of comparison and was obtained from Sigma Aldrich (lot#062k4698, Germany). Nystatin has been used in some previous studies; however, since C. krusei was also used in this study, we had to use a stronger antifungal agent.

Candida strains were cultured on Mueller Hinton agar culture medium using a sterile loop. The plates were then incubated at 37°C; Candida colonies appeared on the culture medium after 24 hours. Twenty-four-hour cultures of C. albicans and C. krusei on Mueller Hinton agar were used to make fungal suspensions. Fungal suspensions were prepared at 0.5McFarland standard concentration, which was equal to 5×10^8 colony forming units (CFUs)/10 µL. [6] The prepared fungal suspensions were diluted 1/20 using distilled water; 10 µL of the fungal suspension was added to the wells and the plates were incubated at 37°C for 24 hours. Thus, each well contained 5×10^8 CFUs/10 µL of the microbial suspension.

In order to prepare serial dilutions of amphotericin B according to the protocol provided by the Sigma Aldrich, 1cc of 2% dimethyl sulfoxide (DMSO) was added to 5mg of amphotericin B to obtain 5mg/mL concentration of amphotericin B. Using the same method, serial dilutions of the extracts were prepared.

The well with minimum concentration of extract that inhibited fungal growth, and consequently showed the minimum number of grown colonies in the Mueller Hinton agar medium was considered as the MIC and no fungal growth was observed in the presence of the next dilute of solution. [7] According to Diba et al. 24 hours were required in order to see the effect of MIC of extract on fungal growth; thus, we allowed 24 hours for the extract to take effect . [8]

Results

This study assessed the antifungal effects of different concentrations of aqueous and ethanolic extracts of cinnamon in 2% DMSO solvent on C. albicans and C. krusei using the MIC method. All tests were repeated in triplicate. To ensure that DMSO solvent had no adverse effects on the fungi, the highest concentration of DMSO without the extract was considered as the drug control and a test tube containing culture medium without the fungi was considered as the test control.

C.albicans: Ethanolic extract of cinnamon at 50mg/mL concentration and aqueous extract of cinnamon at 25 mg/mL concentration inhibited the growth of C. albicans, while amphotericin B prevented the growth of C. albicans at 0.015 mg/mL concentration.

C.krusei*:* Ethanolic extract of cinnamon at 50mg/mL concentration and aqueous extract of cinnamon at 100 mg/mL concentration inhibited the growth of C. krusei. On the other hand, amphotericin B prevented the growth of C. krusei at 0.015 mg/mL concentration.

The MIC of the extracts of cinnamon was different from that of amphotericin B for Candida strains. The three samples of each strain and extracts showed the same MIC values; in other words, no fungal growth was noted and the MIC values of the groups did not show any variation in the three samplings. Thus, no statistical test was used. If there were variations in at least one of

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the three samples taken of each strain and extract (in case of variability of MIC values) a statistical test would be used for the purpose of comparison with other groups.

Discussion

The results of this study showed that ethanolic extract of cinnamon in 50 mg/mL concentration inhibited the growth of C. albicans and C. krusei. Aqueous extract of cinnamon prevented the growth of C. albicans in 25 mg/mL concentration and the growth of C. krusei in 100 mg/mL concentration, whereas amphotericin B at 0.015 mg/mL concentration prevented the growth of C. albicans and C. krusei.

Arbabi et al. assessed the antimicrobial efficacy of thyme, clove and cinnamon extracts against C. albicans in comparison with that of nystatin. The diameter of the growth inhibition zones caused by each extract was compared with that of the positive control group. Nystatin had the highest antifungal efficacy followed by cinnamon, cloves and thyme (P<0.000). They concluded that thyme, clove and cinnamon had significant antifungal effects on C. albicans. [9] Their findings regarding the favorable antifungal effects of cinnamon were in agreement with our findings although the method of assessment of the antifungal efficacy of cinnamon extract was not the same in the two studies.

Atai et al. in their in vitro study compared the antifungal efficacy of wormwood, eucalyptus, onion, cinnamon, turmeric, sage, mint and evergreen extracts against standard strains of C. albicans compared to nystatin mouthwash. The results showed that all six extracts had antifungal activity but the antifungal efficacy of turmeric, eucalyptus, wormwood and cinnamon was more significant than that of others. They concluded that these materials had acceptable antifungal efficacy compared to nystatin. [10] Their findings with regard to the acceptable antifungal efficacy of cinnamon were in accordance with ours although the methodologies of the two studies were slightly different.

Abdolmaleki et al. evaluated the antifungal effects of crude extract of cinnamon on phytopathogenic fungi. The results of antifungal susceptibility testing showed that the cinnamon extracts had favorable antifungal efficacy in different concentrations. The greatest inhibitory effect belonged to the acetone extract of cinnamon. The inhibitory effect of 5 mg/mL of the acetone extract of cinnamon was equal to that of 0.5 mg/mL of Mancozeb antifungal toxin against Rhizoctonia solani, Phytophthora drechsleri and Bipolaris sorokiniana. Their findings confirmed the potential of secondary metabolites of cinnamon for inhibition of fungal pathogens. [5] Similarly, the current study also confirmed the antifungal effects of cinnamon.

Since cinnamon has antifungal properties mostly due to cinnamaldehyde which is a fugocide agent, it can be used as an appropriate option in the treatment of oral candidiasis with no side effects.[3]

Conclusion

Despite some differences in the methodology of studies, almost all previous studies on the antifungal efficacy of cinnamon especially against Candida species confirmed its optimal antifungal efficacy. The results of this study showed that ethanolic and aqueous extract of cinnamon inhibited the growth of C. albicans and C. krusei. More studies are required to further confirm the antifungal efficacy of cinnamon. In case of confirming the antifungal effects of cinnamon, it can be used as a medicinal herb for this purpose.

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Conflict of interest disclosure: The authors state that they have no conflicts of interest.

Authors' Contributions

M.Mehdipour was responsible for the study concept and literature search. N.Gholizadeh contributed to the clinical study, data acquisition and manuscript preparation. M.Sadrzadeh-Afshar carried out the data analysis and manuscript preparation. M.Hakemi involved in the clinical study.

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