Original Article

Antifungal Efficacy of Spice Extracts against *Candida albicans*: An *in vitro* study

Pooja Latti, Subramaniam Ramanarayanan[1], G. M. Prashant[2]

Department of Public Health Dentistry, Annoor Dental College and Hospital, Muvattupuzha, [1]Department of Public Health Dentistry, Indira Gandhi Institute of Dental Sciences, Kothamangalam, Kerala, [2]Department of Preventive and Community Dentistry, College of Dental Sciences, Davangere, Karnataka, India

Abstract

Background: *Candida* species are normal commensals and are isolated intra-orally in 17%–75% of healthy individuals and all debilitated people. Eradication of candidiasis is complicated by the emergence of *Candida* strains that are resistant to the currently used antifungal agents. Plants as remedies are gaining popularity in developed countries. Although many plants have already been investigated against *Candida albicans*, the search is still to find a long-term prevention or cure for oral candidiasis. **Objectives:** The objective of this study was (1) to evaluate the antifungal activity of black pepper, bay leaf, cinnamon, and cumin against *C. albicans* and (2) to determine the minimum inhibitory concentration (MIC) of spice extracts against *C. albicans*. **Materials and Methods:** Spices obtained from the local market were dried and powdered. Solvent extracts were obtained by maceration with methanol followed by filtration and evaporation. The antifungal efficacy was assessed using cup-plate diffusion method followed by the determination of MIC by serial tube dilution technique. Statistical analysis was done using one-way analysis of variance followed by Tukey's *post hoc* test. $P < 0.05$ was considered as statistically significant. **Results:** All the extracts evaluated showed variable degree of inhibition zones against *C. albicans* with cinnamon showing the highest inhibition (49.3 ± 0.52) mm and also with least MIC against *C. albicans* (<0.05 mg/ml). **Conclusion:** These results exhibit the antifungal activity of the spice extracts against *C. albicans*, which may be useful in the treatment of oral candidiasis.

Keywords: Antifungal efficacy, *Candida albicans*, spice extracts

Introduction

In developing countries, microorganisms are frequently a cause of prevailing diseases that present a serious public health issue in a significant segment of the population. The economic crisis, high cost of industrialized medicines, and inefficient public access to medical and pharmaceutical services, in addition to the side effects caused by synthetic drugs, are some of the factors contributing to the central role of medicinal plants in health care.[1] *Candida* species are responsible for a wide range of systemic as well as superficial opportunistic infections (candidiasis) occurring most frequently in the vaginal or oral mucosa.[2]

High degrees of antifungal resistance have been reported in *Candida* species, and these have exhibited primary resistance patterns toward drugs such as nystatin, clotrimazole, fluconazole, itraconazole, and amphotericin B.[3] In the present scenario, an emergence of multiple drug resistance in human pathogenic fungi and the small number of antifungal classes of drugs available stimulated research directed toward the discovery of novel antifungal agents from other sources, such as medicinal plants.[1]

For thousands of years, various natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. According to the WHO, medicinal plants would be the best source for obtaining a variety of drugs.[4]

Furthermore, herbal products have been in use since ages for treatment of gum and tooth-related problems.[5] These evidence quantify and highlight the importance of utilizing natural products for the betterment of oral health.

Access this article online

Quick Response Code:

Website: www.ijcm.org.in

DOI: 10.4103/ijcm.IJCM_140_19

Address for correspondence: Dr. Pooja Latti, Department of Public Health Dentistry, Annoor Dental College and Hospital, Perumattom, Muvattupuzha - 686 673, Kerala, India.
E-mail: drpooja_l@yahoo.co.in

How to cite this article: Latti P, Ramanarayanan S, Prashant GM. Antifungal efficacy of spice extracts against *Candida albicans*: An *in vitro* study. Indian J Community Med 2019;44:S77-80.

Received: 03-04-19, **Accepted:** 29-08-19

Thus, the objectives of the present study were to investigate the *in vitro* antifungal activity of black pepper, Indian bay leaf, cinnamon, and cumin and to determine the minimum inhibitory concentration (MIC) of the spice extracts against *Candida albicans*.

Materials and Methods

Preextraction preparation

Cinnamon bark, cumin, and dried black pepper fruits and dried Indian bay leaves were obtained from the local market of Davangere city, Karnataka, India. The spices were cleaned and washed with sterile distilled water, air-dried on filter paper at room temperature, and powdered using a grinder. The powder was passed through a sieve to maintain uniform size.

Extract preparation

Solvent extracts were prepared with methanol by maceration technique. Ten grams of ground spice was added to 100 ml methanol and agitated at room temperature for 8 h. The mixture was allowed to stand for 12 h and subsequently concentrated by complete evaporation of solvent at room temperature to yield pure extracts. Pure solutions of crude extracts with 100% concentration were prepared by mixing the appropriate amount of extracts with an inert solvent di-methyl sulfoxide (negative control). For the determination of MIC s appropriate dilutions of the extracts were with the inert solvent.

Test-microorganism

The standard strain of *C. albicans* used in this study was ATCC 2091. The organism was sub-cultured on Sabouraud's Dextrose agar at 37°C for 48 h, and the stock culture was maintained at 4°C.

Screening for antifungal activity

Cup plate diffusion method

The antifungal activity of the spice extracts was assessed by the cup plate diffusion method.[6] Petri dishes containing 18 ml of Sabouraud's dextrose agar for *C. albicans* were poured. One hundred microlitres of inoculum of each test organism was spread onto the specific media plates to achieve a confluent growth. Wells or cups of 8 mm diameter were made. One hundred microlitres of each extract was propelled directly into the wells of the inoculated plates, allowed to stand for 10 min for diffusion, and incubated at 37°C for 24 h. The experiment was performed in triplicate ensure consistency.

After incubation for 24 h at 37°C, the plates were observed for antifungal activity by zone of inhibition. The zone of inhibition was measured using vernier calipers and expressed in millimeters.

Determination of minimum inhibitory concentration

The MIC is the lowest concentration capable of inhibiting the growth.[7] In the present study, MIC was determined using "Serial tube dilution technique." In this technique, the tubes of broth medium, containing graded doses of compounds are inoculated with the test organisms. After suitable incubation, growth will occur in those tubes where the concentration of compound is below the inhibitory level and the culture will become turbid (cloudy). Therefore, growth will not occur above the inhibitory level, and the tube will remain clear.

Statistical analysis

Data collected by experiments were computerized and analyzed using the SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc. Mean and standard deviation was calculated. One way analysis of variance followed by Tukey *post hoc* test was used. $P < 0.05$ was considered statistically significant.

Results

All the extracts evaluated showed variable degree of inhibition zones against *C. albicans*. Significant differences in the mean zone of inhibition observed among all the four spice extracts. Cinnamon extract showed the maximum antifungal activity with zone of inhibition of 49.3 ± 0.52 mm, followed by cumin (44.7 ± 0.37 mm), bay leaf (15.9 ± 0.34 mm) and black pepper (13.9 ± 0.38) [Table 1].

Results of the MIC are summarized in Table 2 and among all the four extracts, cinnamon was seen to have the least MIC (<0.05 mg/ml) against *C. albicans* followed by cumin (0.1 mg/ml).

Discussion

Plant essential oils and extracts have been used since many thousands of years, in food preservation, pharmaceuticals, alternative medicine, and natural therapies. Thus, It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare.[8] Several investigations have been directed towards their antibacterial properties.[9] Not many studies investigating the antifungal efficacy of spice extracts have been reported. The present study gives an account on the antifungal activity of solvent extracts of cinnamon, black pepper, cumin, and bay leaf against *C. albicans*. These spices have been routinely used in Indian kitchens since ages.

Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of candidal species, the most common being *C. albicans*. The underlying causes of oral candidiasis include extremes of age, xerostomia, antibiotic therapy, dentures, smoking, Cushing syndrome, malignancies, immune deficiencies, and diabetes mellitus.[10]

Organic solvent (methanol) rather than distilled water was used in the preparation of the extracts. The polarity of antibacterial compounds make them more readily extracted by organic solvents, and using organic solvents does not negatively affect their bioactivity against bacterial species.[11]

Cinnamon demonstrated excellent antifungal activity against *C. albicans*. The antifungal activity of cinnamon may be attributed to the presence of cinnamaldehyde that inhibits amino acid decarboxylase activity. Cinnamon bark is rich in

Table 1: Antifungal activity of the extracts on *Candida albicans* determined by cup plate diffusion method on specific media

Spice extracts	Mean zone of inhibition (mm)	SD	ANOVA	Tukey's *post hoc*
Cinnamon	49.3	0.52	F=24381.80 P<0.001 (significant)	1>3>4>2
Black pepper	13.9	0.38		
Cumin	44.7	0.37		
Bay leaf	15.9	0.34		

ANOVA: Analysis of variance, SD: Standard deviation

Table 2: Minimum inhibitory concentration values of the extracts against *Candida albicans*

Name of extracts	MIC (mg/ml)
Cinnamon	<0.05
Black pepper	6.25
Cumin	0.1
Bay leaf	0.8

MIC: Minimum inhibitory concentration

cinnamaldehyde (50.5%), which is highly electronegative, which interferes in biological processes involving electron transport and reacts with nitrogen-containing compounds, inhibiting the growth of microorganisms.[12] Results are in agreement with the studies conducted by Aneja *et al*. in Haryana, India[13] and Khan *et al*. in Aligarh, India.[4]

Black pepper extract exhibited antifungal activity against *C. albicans*. The observed antifungal activity may be attributed to the volatile oil of pepper which has been shown to have antimicrobial activity.[14,15] The results are consistent with previous researches conducted by, Johann *et al*. in 2007,[1] Joe *et al*. in 2009[16] and Sasidharan and Menon in 2010.[15]

Cumin's antifungal activity may be attributable to the presence of cumin aldehyde (16.1%), a compound with known antimicrobial properties, and α-pinene, (11.4%) of *Cuminum cyminum* essential oil.[17] The results were similar to studies conducted by and by Pai *et al*. in 2010,[18] Kamble in India, in 2015[19] and Swapna *et al*. in, India, in 2018.[20]

The antifungal activity of Bay leaf may be attributed to the presence of certain volatile oil components such as cinnamic aldehyde and eugenol.[5] The results of our study are consistent with the studies conducted by Yilmaz *et al*. in 2013.[21]

The rampant use of antifungal therapy in individuals has resulted in the onset of antifungal drug resistance. The evolution of antimicrobial drug resistance is an almost inevitable process that is universal in the microbial world. Although fungal resistance is not as rampant as bacterial resistance, the economic facets associated with fungal infections is unacceptably high. Also considering the limited number of antifungal drugs available, one of the main strategies of improving therapy in mycoses is overcoming antifungal resistance.[22]

One of the keys to manage the emerging widespread problem of drug resistance is to develop newer drugs with newer targets, with decreased drug interactions, increased safety and tolerance and with cost-effectiveness.

There has been a constant quest for identifying natural and plant products for the potential use of antimicrobials. The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for the treatment of infectious diseases.[23]

Such products, like the ones analyzed in this study, have a long traditional history of use in India and is accessible, acceptable, affordable, and beneficial to the society at large. This study illustrates the need for modern medicine and science to turn its attention to the plant world once again to find a new medicine that might cure various diseases and conditions in a safe way. The need of the hour is to conduct research on screening of the natural products or plant parts that form the primary platform for further phytochemical and pharmacological studies that may open the possibilities of finding new clinically effective antifungal and antibacterial compounds against the oral pathogens. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. The active ingredients from these spices can be isolated and studied further for their use in dentistry.

Conclusion

All the four extracts showed significant antimicrobial activity with cinnamon demonstrating maximum activity and also with the least MIC against the tested organisms. The current study supports the traditional advantages of the studied spices and suggests their effectiveness.

The study also paves a way for further research on the effects of spices on the microorganisms. Additional *in vivo* studies and clinical trials are widely recommended to justify and further evaluate the potential safety and efficacy of the spices to be used as antimicrobial agents in topical or oral applications.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Johann S, Pizzolatti MG, Donnici CL, Resende MA. Antifungal properties of plants used in Brazilian traditional medicine against clinically relevant fungal pathogens. Braz J Microbiol 2007;38:632-7.
2. Wyk CV, Botha FS, Steenkamp V. *In vitro* antimicrobial activity of medicinal plants against oral *Candida albicans* isolates. Int J Biomed Pharm Sci 2009;3:26-30.
3. Panda SK, Dubey D, Dutta SK. Anticandidal activity of *Diospyros melanoxylon* Roxb. Bark from Similipal biosphere reserve, Orissa, India. Int J Green Pharm 2010;4:102-7.
4. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, *et al.* Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules 2009;14:586-97.
5. Adejumobi JA, Ogundiya MO, Kolapo AL, Okunade M. Phytochemical composition and *in vitro* antimicrobial activity of *Anogeissus leiocarpa* on some common oral pathogens. J Med Plants Res 2008;2:193-6.
6. Gavin JJ. Analytical microbiology. II. The diffusion methods. Appl Microbiol 1957;5:25-33.
7. Mishra N, Behal KK. Antimicrobial activity of some spices against selected microbes. Int J Pharm Pharm Sci 2010;2:187-96.
8. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antibacterial activity of some plant essential oils. BMC Complement Altern Med 2006;6:39.
9. Chaudhry NM, Tariq P. *In vitro* antibacterial activities of kalonji, cumin and poppy seed. Pak J Bot 2008;40:461-7.
10. Subramaniam R, Mittal S, Hiregoudar M, Ratan GN, Sakeenabi B, Prashant GM, *et al*. Antimicrobial activity of stevioside on periodontal pathogens and *Candida albicans* an *in vitro* study. J Indian Assoc Public Health Dent 2011;17 Suppl 1:325-9.
11. Goyal P, Chauhan A, Kaushik P. Laboratory evaluation of crude extracts of *Cinnamomum tamala* for potential antibacterial activity. Electron J Biol 2009;5:75-9.
12. Gupta C, Garg AP, Uniyal RC, Kumari A. Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes. Afr J Microbiol Res 2008;2:247-51.
13. Aneja KR, Joshi R, Sharma C. Antimicrobial activity of dalchini (*Cinnamomum zeylanicum* bark) extracts on some dental caries pathogens. J Pharm Res 2009;2:1387-90.
14. Dorman HJ, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J Appl Microbiol 2000;88:308-16.
15. Sasidharan I, Menon AN. Comparative chemical composition and antimicrobial activity of berry and leaf essential oils of *Piper Nigrum* L. Int J Biol Med Res 2010;1:215-8.
16. Joe MM, Jayachitra J, Vijayapriya M. Antimicrobial activity of some common spices against certain human pathogens. J Med Plants Res 2009;3:1134-6.
17. Iacobellis NS, Lo Cantore P, Capasso F, Senatore F. Antibacterial activity of *Cuminum cyminum* L. And *Carum carvi* L. Essential oils. J Agric Food Chem 2005;53:57-61.
18. Pai MB, Prashant GM, Murlikrishna KS, Shivakumar KM, Chandu GN. Antifungal efficacy of *Punica granatum*, *Acacia nilotica*, *Cuminum cyminum* and *Foeniculum vulgare* on *Candida albicans*: An *in vitro* study. Indian J Dent Res 2010;21:334-6.
19. Kamble VA. *In vitro* anti-fungal activity of *Cuminum cyminum* (cumin seed) essential oil against clinical isolates of *Candida* species. Am J Phytomed Clin Ther 2015;3:264-75.
20. Swapna S, Sulthana IS, Savithri, Mungara M, Manipal S, Rajmohan M, *et al*. Comparative evaluation of the antifungal efficacy of six different plant extracts and their synergistic effect against *Candida albicans*: An *in vitro* study. SRM J Res Dent Sci 2018;9:13-6.
21. Yilmaz ES, Timur M, Aslim B. Antimicrobial, antioxidant activity of the essential oil of bay laurel from Hatay, Turkey. J Essent Oil Bearing Plants 2013;16:108-16.
22. Pai V, Ganavalli A, Kikkeri NN. Antifungal resistance in dermatology. Indian J Dermatol 2018;63:361-8.
23. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.