Efficacy of Clotrimazole, Chlorhexidine, and Tea Tree Oil against Candida albicans: An in vitro Study

UMESH WADGAVE, NAGESH LAKSHMINARAYAN

ABSTRACT

Background: Candida species are an important cause of opportunistic infection in the oral cavity of immunocompromised patients. Various antifungal agents have been used for the treatment of oral candida infection. Tea tree oil is a herbal product which is known to have broad antifungal properties.

Objective: To compare the antifungal efficacy of 1% tea tree oil, 1% clotrimazole, and 0.2% chlorhexidine mouthwashes against Candida albicans.

Materials and methods: Cup plate diffusion method was employed to test antifungal efficacy of three commercially available mouthwashes, namely tea tree oil, clotrimazole, and chlorhexidine on C. albicans strain (ATCC 2091). The maximum zone of inhibition was measured at 48 hours to assess the antifungal potency.

Results: The zone of inhibition of both tea tree oil and chlorhexidine were found to increase with increase in the volume of the agents.

Conclusion: One percent clotrimazole and 0.2% chlorhexidine showed better antifungal efficacy against C. albicans compared with 1% tea tree oil.

Keywords: Antifungal, Clotrimazole, Tea tree oil.

INTRODUCTION

Oropharyngeal candidiasis (OPC) is a frequent problem among immunocompromised and elderly populations. Oropharyngeal candidiasis is a complex mixture of yeasts and hyphae surrounded by extra polymeric matrix material, which encases the cells within to form an impermeable barrier to host defenses and antimicrobial therapy. The main reason for the high incidence of OPC is due to multiplicity of predisposing factors, which aids and abets the conversion of commensal Candida to a parasitic existence. The clinical manifestations include pseudomembranous and erythematous forms, causing symptoms, such as pain, burning sensation, and altered taste; subsequently leading to nutritional compromise.

Various antifungal agents have been used for the treatment of OPC: commonly used ones are topical clotrimazole, chlorhexidine mouthwash, and tea tree oil. Chlorhexidine mouth rinse has potent antifungal activity both in vitro and in vivo. Tea tree oil has also shown topical antifungal activity, with recent clinical data indicating its efficacy in the treatment of dandruff and oral candidiasis. A study has identified that most of the components of tea tree oil have activity against Candida albicans and other fungi. However, no single drug can be regarded as the classic treatment modality and there exists an ambiguity regarding the difference in efficacy of these drugs. Therefore, the aim of the current study was to compare the antifungal efficacy of 1% tea tree oil with 1% clotrimazole and 0.2% chlorhexidine mouth rinses against C. albicans through in vitro study.

MATERIALS AND METHODS

In this in vitro study, the antifungal efficacy of clotrimazole, chlorhexidine, and tea tree oil mouth rinses against C. albicans (ATCC 2091) was assessed and compared.

Retrieving Viable Growth from Freeze-dried Form of Microbes

Nutrient broth was prepared according to the manufacturer’s instructions. This was used to get the viable growth of microbes. After autoclaving the broth, it was cooled to 37°C. A total of 7 mL of this broth was poured into each test tube. Freeze-dried form of microbes was added to it under strict sterile conditions. The test tubes were incubated at 37°C for 24 hours. Turbidity in the test tube confirmed the growth of microbes. Comparison of this turbidity was made with McFarland 0.5 turbidity standard.
Cup Plate Diffusion Method for Testing the Antifungal Properties

Cup plate diffusion method was used to evaluate the antifungal potential of the extract. Petri dishes containing Sabouraud’s dextrose agar for C. albicans was inoculated with approximately 100 µL of microbial strain using swab technique. The agar plates were allowed to dry and wells or cups of 8 mm diameter were prepared with a sterile standard device. A 100 µL volume of each extract was propelled directly into the wells of the inoculated specific media agar plates. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 48 hours.

After incubation for 48 hours at 37°C, the plates were examined for the presence of clear zones of growth of inhibition surrounding the wells pointing their efficacy against C. albicans. Antifungal activity of the drugs were determined by measuring the zones of inhibition in millimeter for the fungal isolates. Zones of microbial inhibition around the wells containing the test material were measured with a pair of vernier calipers and recorded after the incubation. The shortest distance (mm) from one point of microbial growth to another around the well was considered as the “zone of inhibition.” (The diameter of zones of inhibition includes the diameter of each well, 6 mm, as well). The measurements were repeated four times and their mean was taken as the final measurement. The size of the zone of inhibition determined the effectiveness of the drugs in inhibiting the C. albicans growth. All assays were performed under aseptic conditions and the experiment was repeated three times using three separate culture plates with inocula derived from the same initial pure culture.

Statistical Analysis

All the data were entered into Microsoft Excel Spreadsheet – 2007 version and then imported to Statistical Package for the Social Sciences (SPSS) version 16.0. Data were expressed as mean ± standard deviation.

RESULTS

The zone of inhibition for 1% tea tree oil, 1% clotrimazole, and 0.2% chlorhexidine mouth washes against C. albicans was measured in millimeters (mm). Both tea tree oil and chlorhexidine showed an increase in the zone of inhibition as the volume of the solution was increased. Candida albicans showed resistance to both the agents at lower volumes, whereas for clotrimazole, the zone of inhibition was more than 40 mm at all concentrations (Table 1); one tea tree oil and 0.2% chlorhexidine showed 12 and 15 mm of zone of inhibition at 75 µL concentration respectively.

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<th>Table 1: Zone of inhibition of antifungal agents against Candida albicans</th>
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<td>1% tea tree oil</td>
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<td>chlorhexidine</td>
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<td>1% clotrimazole</td>
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<td>R: Resistant</td>
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DISCUSSION

Candida albicans is the most frequent fungal species isolated from human infections (80–90%) and is responsible for 75% of neonatal infections. Occurrence of oral candidiasis may be considered a potential risk for the occurrence of systemic disease especially among immunocompromised patients. The ability of C. albicans to exist within complex biofilm consortia provides protection from mechanical forces, and the biofilm polymeric matrix provides a barrier of diffusion to antifungal agents. Therefore, infections caused by Candida biofilms are frequently resistant to a range of treatment regimens.

The interest in nonconventional, nonprescription natural medicines in the field of infection parallels an increased awareness of side effects of conventional drugs. There is also a necessity finding new approaches in the therapy of infections in an era of emerging and remerging infections and the spread of antimicrobial drug resistance. Recently, essential oils and their components distilled from vegetable materials have been found to express antimicrobial, antioxidant, pharmacological, and anticancer activities. Among the essential oils, Australian tea tree oil is the most important, because it demonstrated a broad spectrum of biological activities. Terpinen-4-ol is the major tea tree oil component and has shown strong antimicrobial and anti-inflammatory properties. Tea tree oil had a significant curative effect on experimental vaginal candidiasis in rats.

The medicinal uses of tea tree oil relate primarily to its anti-inflammatory and antimicrobial properties. It’s a topical antimicrobial agent is supported by a growing body of clinical data indicating that tea tree oil is effective in the treatment of infections or conditions such as herpes labialis, acne, tinea, onychomycosis, and oral candidiasis. The three major components of tea tree oil are terpinen-4-ol, c-terpinene, and a-terpinene. Previous studies have already proven the effectiveness of tea tree oil against oral candidiasis. The present study compared the in vitro antifungal efficacy of tea tree oil with already established antifungal agents 1% clotrimazole and 0.2% chlorhexidine.

Clotrimazole is a highly effective antifungal agent against mucosal C. albicans infections. Clotrimazole is one of the most commonly used imidazoles for the
treatment of candidiasis. Furthermore, clotrimazole have been shown to affect *C. albicans* farnesol production and to reduce virulence *in vivo*. Clotrimazole acts against fungi by inhibiting ergosterol synthesis. Inhibition of ergosterol synthesis leads to structural and functional impairment of the fungal cytoplasmic membrane. It has a broad antymycotic spectrum of action *in vitro* and *in vivo*.

Chlorhexidine gluconate is a cationic biguanide with broad spectrum antimicrobial action. Literature regarding chlorhexidine has generated interest based not only on its antimicrobial, but also on its antifungal activity. Chlorhexidine gluconate in a concentration of 0.2% is widely prescribed in dentistry as an antiseptic mouth wash due to its broad spectrum antimicrobial activity including *C. albicans*. The antifungal effect of chlorhexidine has been demonstrated in several *in vivo* and *in vitro* trials including some related to *Candida*. It has been shown that exposure of the *C. albicans* isolates to 0.2% chlorhexidine gluconate profoundly suppresses the ability of the former to adhere to buccal cells both in healthy and diseased individuals, such as diabetics.

The present study revealed that tea tree oil was less efficacious against *C. albicans* as compared with the 1% clotrimazole and 0.2% chlorhexidine mouth washes, but 1% clotrimazole showed superior zone of inhibition than 0.2% chlorhexidine. However, in a comparison of the efficacy of fluconazole and miconazole to chlorhexidine against *C. albicans* biofilms, it was shown that chlorhexidine was significantly more effective than the azoles. These variances in results might be due to the different methodology employed in studies. This being an *in vitro* study, extrapolation is limited and further *in vivo* studies are required.

The study results should, however, be interpreted cautiously because the similar antifungal potential of tea tree oil extracts against the tested microorganisms cannot be expected if the microorganisms are present as a part of oral biofilm. Agar diffusion test is a standard method to assess the *in vitro* antimicrobial property of a drug. But, the zone of inhibition of a microorganism by a test agent depends on its diffusion capacity through the solid agar medium. Hence, further *in vitro* studies in biofilm models followed by properly designed randomized controlled trials should be carried out to substantiate the superior antifungal potential of 1% tea tree oil as compared with 1% clotrimazole and 0.2% chlorhexidine.

CONCLUSION

One percent clotrimazole and 0.2% chlorhexidine have better antifungal activity against *C. albicans* compared with 1% tea tree oil. Antifungal efficacy of 1% tea tree oil vanished when the volume was reduced below 50 μL.

ACKNOWLEDGMENTS

Authors would like to thank the faculty of Department of “Molecular Biology and Immunology” of Maratha Mandal’s Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre, Belgaum, for performing chemical assays.

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