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BRIEF REPORT

Antifungal activity and physical characterization of an experimental dentifrice containing carvacrol

Actividad antifúngica y la caracterización física de un dentífrico experimental que contiene carvacrol

Maria Thais Soares de Macêdo[1], Ellen Caroline Araújo da Silva Oliveira[1], Jocianelle Maria Fernandes Félix Nunes[2], Fábio Correia Sampaio[2], Isabela Albuquerque Passos Farias[3] ✉

Resumen

Introducción: El tratamiento exitoso de la candidiasis oral depende de tres principios esenciales, a saber: diagnóstico temprano y preciso, correlación con factores predisponentes o enfermedades subyacentes que comprometan la inmunidad y uso apropiado de medicamentos antimicóticos.

Objetivos: Determinar la concentración inhibitoria mínima de carvacrol contra Candida albicans y desarrollar y evaluar la actividad antifúngica in vitro (diámetro de la zona de inhibición) y las propiedades físicas (capacidad espumante, esparcibilidad y capacidad de limpieza) de un dentífrico experimental que contiene carvacrol.

Métodos: El carvacrol se incorporó a una base dentífrica a diferentes concentraciones y se probó su concentración mínima inhibitoria y difusión en agar contra Candida albicans y las propiedades físicas. Los datos fueron analizados por ANOVA.

Resultados: La concentración mínima inhibitoria de carvacrol fue 1041,67 ± 360,84 µg/mL. El dentífrico con carvacrol C1 y C2 produjo un halo de inhibición de 27,50 ± 2,12 mm y 36,66 ± 2,08 mm, respectivamente (p < 0,05). En cuanto a las propiedades físicas, los dentífricos no mostraron capacidad espumante, mientras que su capacidad de limpieza y esparcibilidad permanecieron inalteradas.

Conclusiones: Los dentífricos experimentales que contenían carvacrol mostraron actividad antifúngica. La incorporación de carvacrol alteró significativamente la capacidad espumante de las formulaciones, sin efectos significativos sobre su capacidad de limpieza y esparcibilidad.

Palabras clave: Candida albicans; dentífricos; candidiasis bucal; aceites volátiles.

Abstract

Introduction: The successful treatment of oral candidiasis depends on three essential principles, namely: early and accurate diagnosis, correlation with predisposing factors or underlying diseases that compromise immunity, and appropriate use of antifungal drugs.

Objectives: To determine the minimum inhibitory concentration of carvacrol against Candida albicans and to develop and evaluate the in vitro antifungal activity (diameter of inhibition zone) and physical properties (foaming capacity, spreadability and cleaning capacity) of an experimental dentifrice containing carvacrol.

Methods: The carvacrol was incorporated into a dentifrice base at different concentrations and tested for its minimum inhibitory concentration and agar diffusion against Candida albicans and the physical properties. Data were analysed by ANOVA.

Results: The minimum inhibitory concentration of carvacrol was 1041.67 ± 360.84 µg/mL. The dentifrice containing carvacrol C1 e C2 produced an inhibition zone of 27.50 ± 2.12 mm and 36.66 ± 2.08 mm, respectively (p<0.05). As for the physical properties, the dentifrices showed no foaming capacity, while their cleaning capacity and spreadability remained unaltered.

Conclusions: The experimental dentifrices containing carvacrol showed antifungal activity. The incorporation of carvacrol significantly altered the foaming capacity of the formulations, without any significant effects on their cleaning capacity and spreadability.

Keywords: Candida albicans; dentifrices; candidiasis, oral; essential oil.

Introduction

The successful treatment of oral candidiasis depends on three essential principles, namely: early and accurate diagnosis, correlation with predisposing factors or underlying diseases (e.g. xerostomia, denture fitting, endocrine disords, antibiotic treatment) that compromise immunity, and appropriate use of antifungal drugs.[1]

Carvacrol (5-isopropyl-2-methyl phenol) is a monoterpene phenol commonly found in the essential oils of aromatic plants,[2] and it is safe for human consumption at low concentrations.[3] The pharmacological properties of carvacrol have been studied, including antibacterial[4] and antifungal activity against C. albicans by inducing the apoptosis.[5]

Thus, considering the antimicrobial efficacy and safety of the topical administration of carvacrol,[3]

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[1]Federal University of Paraiba, School of Dentistry. João Pessoa, Brazil.
[2]Federal University of Paraiba, Department of Clinical and Social Dentistry. João Pessoa, Brazil.
[3]Federal University of Paraiba, Department of Restorative Dentistry. João Pessoa, Brazil.

we reasoned that it could be an effective active ingredient in a dentifrice formulation for the management of oral candidiasis. The null hypothesis tested were: 1) the use of carvacol is not able to reduce C. albicans activity; 2) incorporating carvacrol into a dentifrice base does not have an additional antifungal effect as compared to dentifrice alone; 3) incorporating carvacrol into a dentifrice base does not change the foaming capacity, spreadability and cleaning capacity as compared to dentifrice alone. Hence, the aims of this study were to determine minimum inhibitory concentration of carvacrol against C. albicans and to develop and evaluate the in vitro antifungal activity (diameter of inhibition zone) and physical properties (foaming capacity, spreadability and cleaning capacity) of an experimental dentifrice containing carvacrol.

Methods

An in vitro experimental and cross-sectional study was designed. The analysing laboratory was blinded to experimental dentifrice concentration. This study was approved under protocol PVG12192-2020/ Federal University of Paraíba, Brazil.

Determination of the antifungal activity

The Minimum Inhibitory Concentration (MIC) of carvacrol against C. albicans from the American Type Culture Collection (ATCC 76645) was determined by the 96-well microdilution method.[6] Briefly, carvacrol was obtained from Sigma-Aldrich® (São Paulo, Brazil) and solubilized in 5 % dimethylsulfoxide (DMSO) (Diadema, SP, Brazil) at a 1:1 ratio.

The experimental dentifrice formulations were prepared by incorporating the carvacrol solution into a dentifrice base (Colgate My First, infant, fluoride-free, Colgate-Palmolive Company) under shaking at the MIC (C1) or 2xMIC (C2). The dentifrice base, without carvacrol, was used as the control. The agar diffusion method was carried out with the experimental dentifrices into Sabouraud dextrose agar after the standardization (0.5 McFarland scale) of the yeast inoculum by spectrophotometer.[7] The sensitivity of the fungal strain to the dentifrices was classified based on the diameter of inhibition zones in millimeters.[8]

Physical characterization of the experimental dentifrices

The percent foaming capacity of the blinded experimental dentifrices was determined by measuring the foam height above the water divided by the total height.[9] The spreadability of the dentifrices was verified on a glass plate.[9] The diameter of the diffused sample was measured with a manual caliper. The cleaning capacity of the dentifrices was investigated as previously described[10] using the technique of dye in the eggshell due to the similarity between such surface and the dental enamel, with calcium being the major component thereon.[9]

Statistical analysis

The data collected were submitted to descriptive and inferential statistical analysis using the SPSS (Statistical Package for the Social Sciences) version 20.0 (SPSS Inc., Chicago, IL, USA). After the Shapiro-Wilk normality test, one-way analysis of variance (ANOVA) followed by Tukey's posthoc test was used to detect differences in the inhibition zones (mm) between the groups. A 5 % significance level was considered in the data analysis.

Results

The MIC of carvacrol against C. albicans was 1041.67 ± 360.84 µg/mL. The dentifrice containing carvacrol (C1) produced an inhibition zone of 27.50 ± 2.12 mm, with a statistically significant difference ($p < 0.05$) in relation to C2 (36,66 ± 2.08 mm). Hence, the strain susceptibility to the experimental dentifrice was considered to be "extremely sensitive (+++)" (table 1).

Table 1- *Values of inhibition zone obtained by agar diffusion method of experimental dentifrice containing carvacrol against Candida albicans*

Experimental dentifrice	Concentration of carvacrol (mg/ mL)	Inhibition zone (mm)	Strain susceptibility
C1	1.04	27.50 ± 2.12	(+++)
C2	2.08	36.66 ± 2.08	(+++)
C3	0.0	0,0 ± 0.0	(-)

Data presented as mean ± standard error of the mean of three inhibition zone analyzed by ANOVA with Tukey post test. $p < 0.05$; (-) not sensitive; (+++) extremely sensitive.

Next, we tested the experimental formulations for some physical alterations resulting from the incorporation of carvacrol. The formulations showed 0 % foaming formation but their cleaning capacity and spreadability were unaltered (table 2).

***Table 2**- Physical properties of experimental dentifrice containing carvacrol*

Experimental dentifrice	Concentration of carvacrol (mg/ mL)	Foaming ability (%)	Spreadability (cm)	Cleaning ability
C1	3,12	0	8,9	+
C2	6,25	0	9,2	+
C3	0	18	5,1	+

(+): Present

Discussion

There are several strategies to prevent the onset of oral diseases and facilitate dental care and management. Among these the choice of anti-microbial materials,[11] a correct management of hard tissue and restorative materials' surfaces[12] as well as good oral hygiene[1] are pivotal for a long-term success. In this perspective, the use of a dentifrice containing a natural antifungal agent may be an interesting alternative for the treatment of oral candidiasis, especially if the active ingredient of the dentifrice is a phytoconstituent with proven safety in humans, such as carvacrol.[3]

Our data confirmed the antifungal activity of carvacrol against C. albicans, which justified its incorporation into the dentifrice formulation, even though the MIC values observed herein were greater than those reported elsewhere.[5] The results indicated that carvacrol showed a moderate inhibitory activity.[13]

Interestingly, a higher sensitivity of C. albicans cells was observed upon the incorporation of carvacrol into the dentifrice base, which produced large inhibition zones. Similar inhibition zone diameters were previously observed for herbal and conventional toothpaste against the salivary microflora.[14]

Differently from what was observed for the antifungal activity, the incorporation of carvacrol had a negative influence on the foaming capacity, probably due to the lipophilicity of the molecule, unlike dentifrices containing other phytoconstituents.[15] Nevertheless, the cleaning capacity and spreadability of the dentifrices remained unaltered, which is in line with a similar analysis previously reported.[9]

A limitation of the current study was the inability to test the action of experimental dentifrices on multispecies biofilms, which would reflect more accurately the conditions found in the oral cavity. Hence, further tests to validate the effectiveness of these dentifricies on the multispecies biofilm model would be necessary. In addition, the findings described here encourage the development of clinical trials to evaluate the efficacy of experimental dentifrices containing carvacrol.

Conclusions

The experimental dentifrices containing carvacrol showed antifungal activity against C. albicans. The incorporation of carvacrol significantly altered the foaming capacity of the formulations, without any significant effects on their cleaning capacity and spreadability.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Authorship contribution

Conceptualization: Isabela Albuquerque Passos Farias.
Fund acquisition: Isabela Albuquerque Passos Farias, Fábio Correia Sampaio,
Formal analysis: Isabela Albuquerque Passos Farias.
Research: Maria Thais Soares de Macêdo, Ellen Caroline Araújo da Silva Oliveira.
Methodology: Isabela Albuquerque Passos Farias, Fábio Correia Sampaio, Jocianelle Maria Fernandes Félix Nunes.
Administration: Isabela Albuquerque Passos Farias.
Supervision: Isabela Albuquerque Passos Farias, Jocianelle Maria Fernandes Félix Nunes.
Writing – original draft: Isabela Albuquerque Passos Farias, Jocianelle Maria Fernandes Félix Nunes, Maria Thais Soares de Macêdo, Ellen Caroline Araújo da Silva Oliveira.
Writing – revision and editing: Isabela Albuquerque Passos Farias.