The effect of Rosmarinus officinalis essential oil on germ tube formation in Candida dubliniensis recovered from denture users

O efeito do óleo essencial de Rosmarinus officinalis sobre a formação do tubo germinativo em Candida dubliniensis isolada de usuários de dentadura

El efecto del aceite esencial de Rosmarinus officinalis sobre la formación del tubo germinativo en Candida dubliniensis aislada de usuarios de dentadura

ABSTRACT

Candida dubliniensis is an emerging pathogenic yeast that is closely related to C. albicans and colonizes or infects the oral cavities of denture users. Similar to C. albicans, C. dubliniensis produces germ tubes as a major virulence factor. Due to the scarcity of data demonstrating that natural products have anti-C. dubliniensis activity, the objective of this study was to evaluate the effect of an essential oil from Rosmarinus officinalis on C. dubliniensis germ tube formation. Six isolates of C. dubliniensis were recovered from the oral cavities (n = 5/denture base and n = 1/palatal mucosa) of six denture users either with (n = 4) or without (n = 2) stomatitis. Germ tube formation was induced in Sabouraud dextrose broth containing 10% fetal bovine serum in the presence (test) or absence (control) of 4% R. officinalis essential oil. The complete inhibition of germ tube formation for all of the tested isolates of C. dubliniensis was observed (p = 0.004). Our results demonstrate the potent anti-C. dubliniensis effect of R. officinalis essential oil, indicating the need to further investigate the pharmaceutical use of R. officinalis essential oil, particularly as it impacts the colonization of denture resin and stomatitis.

Keywords: Candida dubliniensis; Rosmarinus officinalis; Stomatitis, Dentures.

INTRODUCTION

Candida dubliniensis has been described by Sullivan et al.1 as a group of atypical isolates of Candida due to the significant genomic differences from C. tropicalis, C. parapsilosis, C. glabrata, C. kefyr, and C. krusei. C. dubliniensis is closely related to C. albicans; both are chlamydospore and germ-tube producing species that are differentiated by the use of a variety of techniques for yeast identification, including the VITEK 2 ID-YST system and differential media2, such as Tobacco agar3. Reports on the isolation of C. dubliniensis show a link to oral infections in HIV-negative and HIV-positive individuals4. C. dubliniensis can cause oral infections in denture users (with or without stomatitis), either in the presence5 or absence6 of C. albicans. The pathogenesis of oral candidiasis due to C. dubliniensis begins with the initial adherence of the organism to mucosal surfaces via a mechanism that directly involves cell surface hydrophobicity and then
switches to pseudohyphae or true hyphae, considered the major virulence factor. Therefore, this species is considered an emerging yeast associated with oral candidiasis, despite being the smallest constituent of the oral microflora in humans. Within this context, studies have demonstrated the effectiveness of compounds (synthetic or natural) for inhibiting the pathogenicity of C. dubliniensis recovered from the oral cavity. Indeed, previous reports have indicated the antifungal activity of the essential oils of medicinal plants against Candida spp., including compounds from Rosmarinus officinalis. Therefore, the aim of the present study was to evaluate the inhibition of germ tube formation by C. dubliniensis recovered from denture users using R. officinalis essential oil.

MATERIALS AND METHODS

FUNGAL ISOLATES AND GROWTH CONDITIONS

A total of six isolates of C. dubliniensis were recovered from the oral cavities of six denture users (Table 1). The yeasts were recovered from the denture base or palatal mucosa using a sterile swab. Sampling was conducted from March 2012 to October 2012 at the dental school clinic of the Universidade Federal do Pará, Brazil. The study was approved by the Research Ethics Committee of the Instituto Evandro Chagas (CEP/IEC 032/10; January 13, 2011), and all denture users gave informed consent. The isolates were identified from a carbohydrate assimilation profile using the VITEK 2 system (bioMérieux, Marcy l’Etoile, France). After 3 hours of incubation in fetal bovine serum at 37°C, germ tube formation and the production of wrinkled colonies with the presence of hyphae and chlamydospores in Tobacco agar were used to confirm the presence of C. dubliniensis. The C. dubliniensis isolates were then cultivated in Sabouraud dextrose agar (Difco, Laboratories, Detroit, MI, USA) under aerobic conditions at 37°C for 24 hours. A suspension of $10^6$ cells/mL in sterile (pH 7.2) phosphate-buffered saline (PBS) was produced for each isolate and used to assay the inhibition of germ tube formation.

EXTRACTION OF R. OFFICINALIS ESSENTIAL OIL

R. officinalis was collected (collector: Gauch, L.M.R 01) and identified by Ely Simone Cajueiro Gurgel (Museu Paraense Emílio Goeldi, Pará State, Brazil) under registration code MG 204.248. The essential oil was obtained from fresh leaves (350 g) by steam distillation for 240 minutes using the Clevenger system. In this process, 2 mL of the essential oil was obtained and subsequently stored under cool, dark conditions. The experiments were conducted using the pure essential oil without fractionation.

GERM TUBE INHIBITION TEST

The effect of R. officinalis essential oil on germ tube formation was evaluated as described by Bernardes et al. Briefly, germ tube formation was induced in Sabouraud dextrose broth containing fetal bovine serum (10%) and the essential oil from R. officinalis (4%) (assay tube) or broth alone (control tube). The yeast suspension (100 µL) was inoculated, and the assay was performed at 37°C for 3 hours. The total cell number was determined using a Neubauer chamber, and germ tube formation was expressed as the percentage of germ tube-forming cells relative to the total number of cells.

STATISTICAL ANALYSIS

The data were analyzed using BioEstat version 5.3 (Mann-Whitney, Kruskal-Wallis, and Fisher exact tests), with $p \leq 0.05$ considered to be statistically significant.

RESULTS

The percentage of cells from each isolate tested, forming germ tube ranged from 53% to 100% (Figure 1), with no significant difference indicated by either the variation in germ tube formation ($p = 0.06$) or the presence or absence of stomatitis ($p = 0.47$) (Table 1, Figure 1). Following the exposure to 4% R. officinalis essential oil, a complete inhibition of germ tube formation was observed for all isolates of C. dubliniensis tested ($p = 0.004$) (Figure 1). Figure 2 illustrates the morphology observed during the germ tube inhibition assay.

Table 1 – Summary data of denture users from whom C. dubliniensis were recovered

<table>
<thead>
<tr>
<th>Number</th>
<th>Gender</th>
<th>Age</th>
<th>Specimen from</th>
<th>Stomatitis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>67</td>
<td>Denture</td>
<td>Without stomatitis</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>52</td>
<td>Denture</td>
<td>Without stomatitis</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>46</td>
<td>Denture</td>
<td>Type II</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>63</td>
<td>Denture</td>
<td>Type III</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>58</td>
<td>Palatal Mucosa</td>
<td>Type I</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>58</td>
<td>Denture</td>
<td>Type I</td>
</tr>
</tbody>
</table>

* Stomatitis type according to Newton’s classification; M: Male; F: Female.
DISCUSSION

In the present study, six isolates of C. dubliniensis recovered from the oral cavities of denture users were tested, reflecting the low frequency of this species in the microbiota of the individuals included in the study. This finding is corroborated by studies of C. dubliniensis isolated from the oral cavity and from denture users presenting with stomatitis\(^5,6\). Although the pathogenesis of oral infection due to C. dubliniensis is poorly understood, germ tube formation is the primary virulence factor that triggers yeast proliferation during the infection process\(^7,18\). Depending on the isolate, different percentages of cells forming germ tubes were observed (Figure 1, \(p = 0.06\)), variation that might be related to variability in the adaptation of each isolate to the incubation conditions in the test. Indeed, the development of hyphae by C. dubliniensis depends on the association between the activation level of transcription factor Crz1 and the composition of the medium that induces filamentation\(^19\). The results from the inhibition of germ tube formation assay observed in this study agree with those described by Pozzatti et al\(^20\), reinforcing the need for the evaluation of pharmaceuticals based on the essential oil of R. officinalis.

Additionally, the reduction in C. dubliniensis colonization of denture resins has been described in an assay using sub-therapeutic concentrations of chlorhexidine gluconate (0.005% and 0.0025%) that are capable of reducing the hydrophobicity of the yeast cell surface\(^11\) and inhibiting the formation of germ tubes\(^20\), thus contributing to the reduction of in vivo pathogenicity. Such reduced pathogenicity is due
essential oil as inhibitors of denture resin
sobre la formación del tubo
was
C. Dubliniensis
tested. Our
23,
C.
22
essential oil, the present
sobre a formação do tubo
Complete inhibition of the formation of germ tube for all isolates of C. dubliniensis tested. Our data demonstrate the ability of this oil to potently inhibit C. dubliniensis, which is important considering the lack of data showing the activity of natural products against this species. Given that four of the six C. dubliniensis isolates tested in this study were recovered from cases of stomatitis (Table 1) and that all were susceptible to the inhibition of germ tube formation in the presence of this essential oil (Figure 1), it is imperative to study this oil more thoroughly. It will be necessary to investigate the applicability of pharmaceutical preparations containing R. officinalis essential oil as inhibitors of denture resin colonization and its effectiveness in treating stomatitis caused by this species of Candida.

CONCLUSION
The present study describes the complete inhibition of germ tube formation in six isolates of C. dubliniensis by exposure to 4% R. officinalis essential oil. The development of pharmaceutical preparations containing this essential oil and the assessment of their potential for the treatment of stomatitis associated with C. dubliniensis are suggested.

CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

O efeito do óleo essencial de Rosmarinus officinalis sobre a formação do tubo germinativo em Candida dubliniensis isolada de usuários de dentadura

RESUMO
Candida dubliniensis é uma levadura patogênica emergente, muito semelhante à C. albicans, que coloniza ou infecta as cavidades bucais de usuários de dentaduras. Similar à C. albicans, C. dubliniensis produz tubos germinativos com fator de virulência maior. Devido à escassez de dados, demonstrando que produtos naturais têm atividade anti-C. dubliniensis, o objetivo deste estudo foi avaliar o efeito de um óleo essencial de Rosmarinus officinalis sobre a formação de tubo germinativo em C. dubliniensis. Seis isolados de C. dubliniensis foram coletados de cavidades orais (n = 5/base da dentadura e n = 1/mucosa palatal) de seis usuários de dentaduras com (n = 4) ou sem (n = 2) estomatite. A formação do tubo germinativo foi induzida em caldo Sabouraud dextrose, contendo soro fetal bovino a 10% na presença (teste) ou ausência (controle) de óleo essencial de R. officinalis a 4%. Observou-se a completa inibição da formação do tubo germinativo de todos os isolados de C. dubliniensis (p = 0,004). Nossos resultados demonstraram o potente efeito anti-C. dubliniensis do óleo essencial R. officinalis, indicando a necessidade de mais investigações sobre o uso farmacêutico do óleo essencial R. officinalis, particularmente o seu impacto sobre a colonização da resina para dentaduras e a estomatite.

Palavras-chave: Candida dubliniensis; Rosmarinus officinalis; Estomatite; Dentaduras.

El efecto del aceite esencial de Rosmarinus officinalis sobre la formación del tubo germinativo en Candida dubliniensis aislada de usuarios de dentadura

RESUMEN
Candida dubliniensis es una levadura patógena emergente, muy parecida a la C. albicans, que coloniza o infecta las cavidades bucales de usuarios de dentaduras. Similar a C. albicans, C. dubliniensis produce tubos germinativos con un mayor factor de virulencia. Debido a la escasez de datos, demostrando que productos naturales tienen una actividad anti-C. dubliniensis, el objetivo de este estudio fue el de evaluar el efecto de un aceite esencial de Rosmarinus officinalis sobre la formación de tubo germinativo en C. dubliniensis. Se recolectaron seis aislados de C. dubliniensis de cavidades orales (n = 5/base de la dentadura y n = 1/mucosa palatal) de seis usuarios de dentaduras con (n = 4) o sin (n = 2) estomatitis. La formación del tubo germinativo fue inducida en caldo Sabouraud dextrose, conteniendo suero fetal bovino a 10% en presencia (prueba) o ausencia (control) de aceite esencial de R. officinalis a 4%. Se observó la completa inhibición de la formación del tubo germinativo de todos los aislados de C. dubliniensis (p = 0,004). Nuestros resultados demostraron el potente efecto anti-C. dubliniensis del aceite esencial R. officinalis, indicando la necesidad de más investigaciones sobre el uso farmacéutico del aceite esencial R. officinalis, particularmente su efecto sobre la colonización de la resina para dentaduras y la estomatitis.

Palabras clave: Candida dubliniensis; Rosmarinus officinalis; Estomatitis; Dentaduras.
REFERENCES


