

Effects of *Rosmarinus officinalis* essential oil on germ tube formation by *Candida albicans* isolated from denture wearers

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ABSTRACT

Introduction: The aim of this study was to investigate the effects of *Rosmarinus officinalis* essential oil on germ tube formation by *Candida albicans* isolated from denture wearers. **Methods:** Ten *C. albicans* isolates recovered from denture wearers were tested using 10% fetal bovine serum with or without 4% *R. officinalis* essential oil. **Results:** The essential oil from *R. officinalis* completely inhibited germ tube formation in the investigated *C. albicans* isolates. **Conclusions:** The results demonstrate that the essential oil of *R. officinalis* modulates *C. albicans* pathogenicity through its primary virulence factor (i.e., germ tube formation was suppressed).

Keywords: *Candida albicans*. Germ Tube. *Rosmarinus officinalis*.

Candida albicans is the primary fungal species associated with oral candidiasis, can adhere to and colonize denture resins, and contributes to denture-related stomatitis, during which the yeast may form germ tubes and hyphae¹. *Candida albicans* virulence depends on its ability to transform between the yeast and filamentous (pseudohyphae or true hyphae) forms²; the latter is induced in human infections³. Recent studies have aimed at developing novel therapies to control denture surface colonization by *Candida* species⁴. Additionally, investigating the antifungal activities^{5,6} of medicinal plants might facilitate the identification of compounds that could potentially be developed into novel antifungal agents. Lima et al.⁷ found that *C. albicans* (ATCC-76615) was sensitive to 8% *Rosmarinus officinalis* essential oil, resulting in a 10-mm inhibition zone. Based on such considerations, the aim of this study was to investigate the effects of *R. officinalis* essential oil on germ tube formation by *C. albicans* isolated from denture wearers.

Ten *C. albicans* isolates were recovered from the oral cavities of 10 denture wearers (Table 1). The yeast was isolated from the denture base or the palatal mucosa using sterile swabs. The samples were collected from March to October 2012 at the School of Clinical Dentistry in the Federal University of

Pará (Brazil). The study was approved by the Research Ethics Committee of the Evandro Chagas Institute (Comitê de Ética em Pesquisa do Instituto Evandro Chagas - CEP/IEC 032/10). The volunteers were informed of the study aims and procedures and signed an informed consent form. The isolates were identified based on their carbohydrate assimilation profiles using the Vitek 2 system (bioMérieux, Marcy l'Etoile, France). Next, the isolates were grown on Sabouraud dextrose agar (Difco, Laboratories, Detroit MI) under aerobic conditions at 37°C for over 24h. A suspension containing 10⁶ cells/ml in sterile PBS (phosphate-buffered saline) (pH 7.2) was generated and used for the germ tube inhibition assay. *Rosmarinus officinalis* was grown in the Jacques Huber botanical garden, which is located at the Coordination of Botany on the research campus of the Goeldi Museum, under 50% shade in black polyethylene pots, with dimensions of 20 × 25cm, filled with black soil substrate; the plants were irrigated as needed to maintain the humidity of the substrate. The specimens were cultured during the rainy season and did not receive any chemical treatment, such as pesticides or fertilizers. The collections of botanic material (by Gauch, L.M.R 01) were held at the growing site, the phenophase of flower buds adults. *R. officinalis* was identified by Ely Simone Cajueiro Gurgel (Emílio Goeldi Museum, Pará, Brazil) using MG 204.248. The essential oil was extracted from fresh leaves (350g) through steam distillation for over 240min using the Clevenger system. The process yielded 2mL of essential oil, which was stored in refrigerated and dark conditions.

The effects of the *R. officinalis* essential oil on germ tube formation were assessed as described by Bernardes et al.⁸ with

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TABLE 1 - Data for the denture wearers who provided the *Candida albicans* isolates.

Patient	Gender	Age	Specimen from	Stomatitis ^a	Germ tube inhibition assay ^b	
					control tube %	test tube %
1	M	62	Denture	Type I	100.0	0.0
2	M	73	Denture	No stomatitis	100.0	0.0
3	F	59	Denture	Type II	100.0	0.0
4	F	68	Mucosa	Type I	100.0	0.0
5	M	63	Denture	Type I	84.0	0.0
6	F	72	Denture	No stomatitis	68.0	0.0
7	M	58	Denture	Type II	63.0	0.0
8	F	54	Denture	Type II	67.0	0.0
9	F	62	Denture	Type I	100.0	0.0
10	F	65	Denture	Type II	94.0	0.0
Positive control					100.0	0.0

^astomatitis type according to Newton's classification. ^bpercentage of germ tubes formed. **M**: male; **F**: female.

some modifications. Briefly, germ tube formation was rapidly induced in Sabouraud dextrose broth using 10% fetal bovine serum and either 4% *R. officinalis* essential oil and 0.02% Tween 80 (assay tube) or nothing (control tube), with 10ml as a final volume. The yeast suspension (100µL) was inoculated, and the test was conducted at 37°C for over 3h. The number of cells was determined using a Neubauer chamber, and germ tube formation was expressed as the percentage of germ tube-forming cells relative to the total cell number. All isolates were tested in duplicate, and the *C. albicans* INCQS 49175 strain was used as a positive control.

The effect of *R. officinalis* essential oil on germ tube formation was assessed in 10 *C. albicans* isolates. In the samples that were treated with 10% fetal bovine serum and no essential oil, the germ tube-forming cells exhibited a range of reactions, including five isolates (5/10) that exhibited 100% germ tube formation; however, the percentage varied from 63% to 94% in the remaining five isolates (5/10). The type of stomatitis exhibited by the denture wearers was not related to the number of germ tube-forming cells. When 4% *R. officinalis* essential oil was added to the incubation, germ tube formation was entirely inhibited in the investigated isolates. All of these results are shown in **Table 1**.

The literature includes many studies assessing the susceptibility of yeasts of the genus *Candida* to medicinal plant derivatives⁶; however, few studies have investigated the plant derivatives' effects on *C. albicans* morphology and germ tube formation. Herein, the complete inhibition of germ tube formation was observed in the investigated isolates when 4% *R. officinalis* essential oil was added. Several studies investigated the antifungal activity of various *R. officinalis* derivatives against *C. albicans*^{5,7}; however, its effect on the primary fungal

virulence factor (i.e., the germ tube) has been neglected. According to Silva et al.⁹, a compound's ability to inhibit germ tube formation could be a means to assess its antifungal activity. In this context, the results of this study clearly demonstrate the antifungal activity of *R. officinalis* essential oil and its capacity to modulate this fungal species' pathogenicity.

Consistent with our results, Bernardes et al.⁸ observed a 95% reduction in germ cell formation when *C. albicans* cells (ATCC 10-231) were treated with raw glycolic 10% (v/v) extract from fresh *Aloe vera* leaves. Interestingly, Pinto et al.¹⁰ demonstrated an effect of the *Ferulago capillaris* essential oil on cells during inhibition of germ tube formation in *C. albicans*, which demonstrated that oxidative stress affects the enzymatic activity as well as cell mitochondrial membrane potential and results in growth inhibition and death. Given the high cost of the required methods, it was impossible to analyze the composition of the essential oil investigated in this study, particularly for the two primary active components, α -pinene and 1,8-cineole¹¹. Knowledge of the essential oil composition is necessary to identify the mechanism underlying the inhibition of germ tube formation in *C. albicans*. Previously, Pozzatti et al.¹² demonstrated that *R. officinalis* extract was able to inhibit germ tube formation in both *C. albicans* and *C. dubliniensis*. Thus, our results were in agreement with this information and illustrated that this essential oil also exhibits antifungal properties.

Because oral mucosa and denture resin colonization by *C. albicans* is a predisposing factor for oral mucosa infections, such as denture-related stomatitis, the ability to reduce yeast viability is a desired quality in candidate products and/or compounds that could be developed into novel therapeutic agents. A study conducted by Ellepola et al.¹³ demonstrated

that sub-therapeutic chlorhexidine chlorhydrate concentrations inhibited germ tube formation in *C. albicans* isolated from the mouth. Gels containing the essential oil from *Pelargonium graveolens*¹⁴ or *Satureja hortensis*¹⁵ induced significant remission of oral mucosa lesions in denture wearers with stomatitis. In this study, treatment with 4% *R. officinalis* essential oil resulted in the full inhibition of germ tube formation in 10 *C. albicans* isolates recovered from denture wearers. Our results provide further evidence that the essential oil of *R. officinalis* is active against *C. albicans* and supports the need to examine the pharmaceutical formulations of this essential oil to treat *C. albicans*-mediated denture-related stomatitis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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