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Red Propolis Antifungal Action on Species of *Candida* of the Oral Cavity

ORIGINAL

Anne Milane Formiga Bezerra[1], Maria do Carmo Andrade Duarte de Farias[2], Kévia Katiúcia Santos Bezerra[2], Francisco Geyson Fontenele Albuquerque[3], Antônio Fernandes Filho[2], Geofabio Sucupira Casimiro[2], Elicarlos Marques Nunes[4], Edivânia Maria Leite da Silva[5], Wilma Kátia Trigueiro Bezerra[1], Palloma Batista de Almeida[1], Tereza Lourença Matias de Araújo[1], Kennia Sibelly Marques de Abrantes[2,6], Luiz Carlos de Abreu[6], Patrício Borges Maracajá[7], Alfredina dos Santos Araújo[7], Rosilene Agra da Silva[7]

Abstract

Introduction: Propolis is a substance that has aroused the interest of many researchers because of its numerous therapeutic properties, antibacterial and antifungal.

Objectives: Identifying the species of *Candida* and evaluate the antifungal effect of red propolis yeast oral cavity.

Method: This is a clinical in vitro study with saliva samples collected from 152 patients treated at the dental office of the Family Health Strategy in the city of São Bento-PB. The identification of *Candida* species was made through the Chrom Ágar *Candida*. The antifungal activity of the propolis extract was analyzed in four different concentrations: 100%, 75%, 50% and 25%, through the agar diffusion test.

Results: The most prevalent species was *C. albicans*; antifungal action as to the concentration of 25% of the propolis extract was that apparently demonstrated greater efficacy, compared to the highest concentration.

Conclusion: The inhibitory effect of propolis against *Candida* may have been influenced by the concentration of alcohol present in the extract. To test this hypothesis suggests that search is performed with extracts of propolis and at the same time with the alcohol, in both concentrations and different environmental conditions. This study offers subsidies for other professionals employ different methodologies and propolis concentrations with other substances in order to test the antimicrobial action of these.

1 Nurse in a Mobile Emergency Service, MSU, Sousa, PB, Brazil.
2 Academic Unit of Life Sciences, Teacher training center, Federal University of Campina Grande.
3 Student of Medicine, Academic Unit of Life Sciences, Federal University of Campina Grande.
4 Faculties Integrated of Patos, PB, Brazil.
5 MSB Visual Communications Ltda – Design and Visual Communication, João Pessoa, PB, Brazil.
6 Scientific Writing Laboratories, Faculty of Medicine of ABC, Santo Andre, SP, Brazil.
7 Federal University of Campina Grande. Graduate-degree in Agroindustrial Systems, Pombal, PB, Brazil.

Contact information:

Maria do Carmo Andrade Duarte de Farias.
Academic Unit of Life Sciences, Teacher training center, Federal University of Campina Grande.

Address: Federal University of Campina Grande. Rua Sérgio Moreira de Figueiredo, sn Bairro Casas Populares, Cajazeiras, PB CEP 58900-000.

carmo.andrade@ufcg.edu.br

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Introduction

Oral Candidiasis is an infection caused by *Candida* yeasts, saprobes microorganisms that, dependent on predisposing factors, become pathogenic [1]. This type of infection is most common in immunocompromised individuals and presented increasing incidence in recent years [2]. *C. albicans* is the most frequently identified species and therefore responsible for this type of infection. However, infections caused by other species of the genus are becoming more frequent, being cited approximately 150 species, most isolated from the oral cavity [3].

C. albicans is considered the main opportunistic pathogenic yeast to be the most frequently isolated species in humans. However, in recent decades there has been a significant increase of other species. The main of medical concern are *C. parapsilosis*, *C. tropicalis, C. krusei, C. guilliermondii, C. glabrata, C. lusitaniae. C. kefyr, C. famata, C. dubliniensis* [4-5].

The species identification is important, fungal infections caused by *Candida*, since the pathogenicity and sensitivity to a particular antifungal vary between different species [6].

The widespread use of azole antifungal for the treatment of oral candidiasis appears to be a major factor in the increased frequency of *Candida* non-albicans species, especially those naturally more resistant to this class of antifungals such as *C. glabrata* and *C. krusei* [7]. Recent studies indicate that there is an increase in resistance of *Candida* species to antifungal drugs available on the market [8].

A drawback of the azole is that they are depending on the concentration, fungistatic, not fungicidal, which may contribute to the development of resistance in clinical isolates [9]. Resistance to azole antifungals may result from target sites of mutation or increased gene expression of proteins responsible for the transport of antifungal to the outside, or even the combination of these two mechanisms, which have been observed in isolated *C. albicans* [10].

This fact has stimulated the search for increasingly new antifungal agents from natural sources and propolis emerges as an important coupled to present a wide diversity of active compounds, many still unknown, with reported biological properties, which may enable the discovery of new bioactive molecules to processes treatments oral disease.

The range of biological activities of propolis is higher in tropical areas of the world, reflecting the plant diversity of these regions [11]. Because of the diversity of the flora, propolises from Brazil were grouped into 12 different groups, according to the chemical composition and biological activities. Currently, a new variety of propolis, originating from the mangrove area in the state of Alagoas (Brazil), had its botanical origin identified as *Dalbergia ecastophyllum*, a kind of legume [12]. This propolis, called "red propolis", because of its intense red coloration, was scored as the 13th type of brazilian propolis and have shown various biological activities *in vitro* assays [13].

Trying to understand the action of propolis, this research was carried out aiming to identify *Candida* species in the oral cavity and evaluate in vitro antifungal activity of the red propolis extract the yeast of the oral cavity.

Material and Method

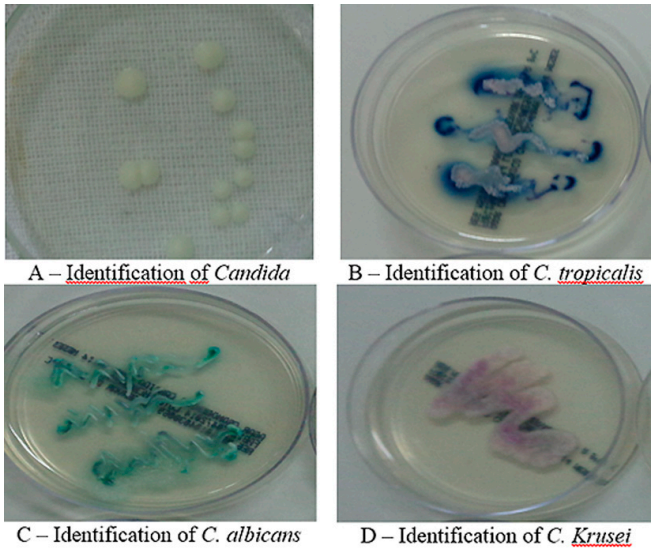
The research is a clinical study *in vitro* with saliva samples collected from 152 patients treated at the dental office of the Family Health Strategy Ivan Olimpo Segundo, in the municipality of São Bentinho-PB, with Oral Swab Sterile aid. The handling of clinical samples was performed at the Laboratory of Vocational Technology Center (VTC) of the Federal University of Campina Grande, PB, Campus Pombal-PB. Patients consented to participate in the survey after they had read, understood and signed two copies of a Free and Clear Informed Consent. In compliance with Resolution 466/12, the research was approved by the Research Ethics Committee of

the Federal University of Campina Grande, CAAE 37184414.5.0000.5575 protocol. Made the collection of oral secretions, identification of the fungus in culture was carried out from the sow Agar Potato Dextrose. The plates were incubated at 37°C for five days. After this period, the growth characteristics of *Candida* colonies were checked. The identification **(Figure 1)** occurred by studying the macroscopic aspects, micro morphological and biochemical in Chrom Ágar *Candida*®. The medium used indicates green colonies, for *C. albicans*; rugose rose, for *C. krusei*; metallic blue, for *C. tropicalis*; and white to violet, for other species [10, 14-15].

The red propolis samples of *Apis mellifera* bees arising from the *Dalbergia ecastophyllum* (Bugiu-of-Tail), whose extract was held to 30% ethyl alcohol PA were acquired in Apiary EDIMEL- João Pessoa - Paraiba.

The antifungal activity of the propolis extract was analyzed in four different concentrations: 100%, 75%, 50% and 25%, determined by Agar diffusion test in which the samples of *Candida* were identified and seeded Agar Potato Dextrose and incubated at 35°C/48h.

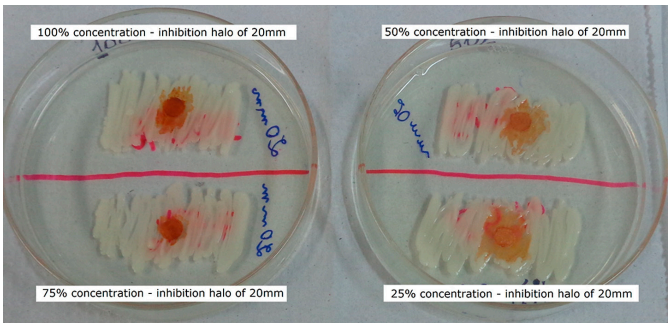
Figure 1: *Candida* species identification in samples of saliva, through the Chrom Agar *Candida*®.



Source: Survey data. São Bentinho-PB, 2014

Then, paper disks were soaked with 10µL used extract concentrations in the three and deposited on the surface of the agar. The plates were incubated at 37°C/48h. After the measurement was performed halos of inhibition of *Candida* growth **(Figure 2)**. From the results, it was considered active product against the species of the microorganisms under study which produced halos that above 10 mm in diameter [16].

Figure 2: Statement of antifungal action of the extract of red propolis at different concentrations – halo of inhibition in 20mm



Source: Survey data. São Bentinho, PB, 2014.

Results

Saliva collection

The result of the salivary culture of 152 participants showed that 27.0% (n = 41) were positive for some species of *Candida*. The data in **Table 1** demonstrate the *Candida* species identified in samples whose culture was positive. Those findings, the species *Candida* albicans prevailed with 68.3% (n = 28), followed of other unidentified species from the culture medium with 14.6% (n = 6).

It was noticed by the findings that half (n = 14) species of *C. albicans* was samples from individuals with clinically healthy mouth, whose reason has been demand service restoration (n = 12) and the dental review (n = 2) (data not shown in tables).

The distribution of *Candida* species according to the gender of the participants, it is clear that in all

Table 1. Species of *Candida* identified in samples of salivary culture (n = 41).

Species of *Candida*	n	%
C. albicans	28	68.3
C. krusei	4	9.8
C. tropicalis	3	7.3
Other species	6	14.6

Source: Survey data. São Bentinho-PB, 2014.

species was greater prevalence in women; the exception of *C. krusei* because the percentages were the same for men and women **(Table 2)**.

Table 2. Distribution of *Candida* species identified in salivary culture according to the gender of the respondents (n = 41).

Species of *Candida*	Gender			
	Woman		Man	
	n	%	n	%
C. albicans (n = 28)	23	82.1	5	17.9
C. krusei (n = 4)	2	50.0	2	50.0
C. tropicalis (n = 3)	3	100.0	0	0.0
Other species (n = 6)	5	83.3	1	16.7

Source: Survey data. São Bentinho-PB, 2014.

In addition to the evidence found on the higher prevalence of *Candida* in women, it was noticed the same fact in participants whose ages ranged from 7 to 30 years, except for *C. krusei*, for all cases met the participants of full age **(Table 3)**.

Interestingly, in this study, cases of *Candida* prevailed in younger patients **(Table 3)** and only 9.7% (4/41) of cases of *Candida* wore partial denture, of which 2 were of *C. albicans* and 2 species, *C. krusei* (data not shown in tables).

Table 3. Distribution of *Candida* species identified in salivary culture according to the age of the respondents (n = 41).

Species of *Candida*	Age (years)			
	7 to 30		31 to 70	
	n	%	n	%
C. albicans (n = 28)	20	71.4	8	28.6
C. krusei (n = 4)	0	0.0	4	100.0
C. tropicalis (n = 3)	3	100.0	0	0.0
Other species (n = 6)	5	83.3	1	16.7

Source: Survey data. São Bentinho-PB, 2014

Antifungal action of the red propolis extract

When testing the antifungal effect of propolis on 41 samples with *Candida* species identified, it was noted that in 61% (n = 25) the result was positive. Analyzing this positivity, it was realized that, except for "other species identified" positive antifungal action prevailed in the other, since the percentages were 64.3% (n = 18) in *C. albicans*, 75% (n = 3) in *C. krusei*, 66.7% (n = 2) in *C. tropicalis* **(Table 4)**.

Table 4. Distribution of *Candida* species identified in salivary culture as antifungal action of red propolis extract (n = 25).

Species of *Candida*	Positive	
	n	%
C. albicans (n = 28)	18	64.3
C. krusei (n = 4)	3	75.0
C. tropicalis (n = 3)	2	66.7
Other species (n = 6)	2	33.3

Source: Survey data. São Bentinho-PB, 2014.

In the above mentioned concentrations of the propolis extract, added to samples containing *Candida* species, it was possible to identify variations from 0 to 60 mm in the inhibition halos. **Table 5** reveals that the average of these halos increased as the concentrations were reduced; 0 mm the percentage reduced, as has reduced the concentration of extract; the positive 60mm was only observed at concentrations of 75% and 25%.

For greater understanding of antifungal action of red propolis extract were excluded lower results than or equal to 10 mm, at all concentrations, as shown in **Chart 1**.

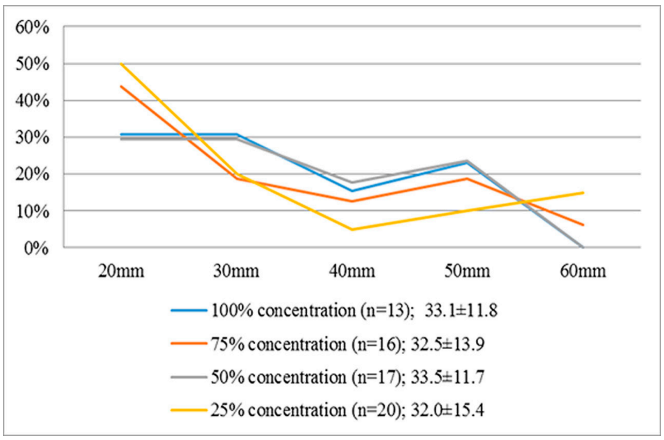
The **Chart 1** indicate that by reducing red propolis extract concentrations were added the cases of positivity antifungal action, from n = 13, concentration 100%, for n = 20, 25% in concentration. This association was not observed in the mean positive

Table 5. Results of the halos of inhibition of antifungal action of the red propolis extract in the concentrations to 100%, 75%, 50% and 25%. (n=25).

Results (mm)	Concentrations of the red propolis extract			
	100%	75%	50%	25%
Average (SD)	18.80±17.64	22.00±18.26	24.00±17.32	26.40±18.00
Minimum-Maximum	0-50	0-60	0-50	0-60
0 mm	32% (n=8)	24% (n=6)	20% (n=5)	12% (n=3)
10 mm	16% (n=4)	12% (n=3)	12% (n=3)	8% (n=2)
20 mm	16% (n=4)	28% (n=7)	20% (n=5)	40% (n=10)
30 mm	16% (n=4)	12% (n=3)	20% (n=5)	16% (n=4)
40 mm	8% (n=2)	8% (n=2)	12% (n=3)	4% (n=1)
50 mm	12% (n=3)	12% (n=3)	16% (n=4)	8% (n=2)
60 mm	-	4% (n=1)	-	12% (n=3)

Source: Survey data. São Bentinho-PB, 2014.

Chart 1: Distribution of antifungal action in mm of red propolis extract at concentrations to 100%, 75%, 50% and 25%.



Source: Survey data. São Bentinho-PB, 2014.

aspects of mergers, because there was almost equivalent concentrations at 100% and 50%; and at concentrations of 75% and 25%.

However, the graphical representation, this trend was not observed in millimetric terms, since there was an increasing linearity in the positivity of antifungal action, as reduced or increased the concentration of propolis extract.

What was observed, on time, it was the highest percentage of 50 mm of positivity found in 23.5% (n = 4) for the concentration of the extract by 50%; and comparing all concentrations, the highest percentage of positivity was 20 mm, equal to 50% (n = 10), referring to the concentration of 25% of the extract.

Discussions

The high prevalence of *C. albicans*, found in this study corroborates the claim that this is the main agent of candidiasis, and this species is 60% of isolates from clinical samples as it is part of human macrobiota [5].

In this sense, scholars [17] found in 82.8% of patients studied in *C. albicans* species. Meanwhile, in another study [18] obtained 62.66% and 66,4%, respectively, of the same species.

About this prevalence, this fact can be justified due to the ability of the *C. albicans* has to produce secreting enzymes such as phospholipase, which plays an important role in the pathogenesis of these yeast [19-21]. The activity of extracellular phospholipase facilitate the capacity of these yeasts to invade host cells and/or processed to remove antigens from the surface of these cells [22].

Contrary to this research, researchers [23] found in the saliva of all samples studied, a frequency of 48% (24/50) of *C. albicans*. That was the most prevalent species in all groups investigated, healthy

or not. However, they identified 47.7% of the *C. albicans* species in patients without oral problems.

Regarding gender, research [24] has shown that, in the sample of 51 women evaluated, 13 (25.5%) had a clinical diagnosis of Vulvovaginal Candidiasis (VVC), and of these, five (9.8%) showed yeasts of the genus *Candida* were also in the oral cavity. The authors observed that from the 15 women without clinical of VVC, 11 (73.3%) showed the yeast in the oral cavity, four (26.7%) in the vaginal mucosa and three (20%) in both sites. In addition, they assessed that although there was no statistically significant correlation between species and sites; women had oral and vaginal candidiasis due to different etiologic agents. Therefore, they should always be advised to maintain good hygiene habits.

The frequency of *Candida* sp. in oral mucosal lesions was identified variable way, by researchers who investigated 832 biopsies of the oral mucosa, of which 27.2% were positive; they confirmed the increased presence of *Candida* in the lesions, being more frequent in males [25].

With regard to age, the *C. albicans* was found in the oral cavity from 3% to 48% of all adults and 45% to 65% of healthy individuals [26]; contradicting the findings in research whose sample consisted of 98.4% adult participants, of which 16.9% reported some pathological episode of oral candidiasis [27].

The relation age and *C. albicans* is reported in a study whose incidence of yeast increased with age, and its presence in the oral cavity, has reached 60% of the edentulous patients over 60 years, who had no signs of injury in the oral mucosa [28].

In recent decades there has been an increasing use of the propolis extracts to assist in the treatment of oral infections, due to their antimicrobial properties.

Different methods in vitro are used for analysis of propolis extracts against species of *Candida* [29]. The antifungal activity of propolis in different species of *Candida* was demonstrated in researches [30], and were also described the effects of the time of year in the activity of Brazilian propolis in *C. albicans* and *C. tropicalis* [31].

The results of this study did not reveal clarity regarding the antifungal effect of red propolis, since the trend of positivity of this action was not growing by increasing or decreasing the concentration of propolis extract. What was observed was that concentration at which 50% was obtained larger inhibition zone, with an average of 33.5 mm (±11.7), then the concentration to 25%, average 32.0 mm (±15.4). On the other hand, analyzing the average and standard deviation of each concentration, it suggested greater efficacy of antifungal activity was the concentration at 25%. These facts raise the possibility that the antifungal action, found in this study, is related to the higher concentration of alcohol added to propolis.

Therefore, studies must be intensified, since research proves natural variations in the chemical composition of propolis extract. In such research, propolis showed excellent antifungal activity, and the concentration was able to inhibit all the yeasts in 5x10-2mg/ml of flavonoids and 2x10-2mg/ml of flavonoids, stimulating cell death, ie 25% and 50% [32].

In a recent study, the activity of propolis produced in Tocantins, in general, showed greater antifungal activity for *C. tropicalis*, with an average of 12 mm of inhibition zone diameter and especially the Palmas-CPI propolis (16 mm). It showed a slightly lower activity for *C. albicans*, average diameter of 11 mm, with the best result corresponding to propolis from Nova Olinda-CPI (15mm) [33].

The in vitro effect of alcoholic propolis extract (APE) produced in different regions, on five species of micro-organisms *(Staphylococcus aureus, Escherichia coli, Enterococcus sp., Pseudomonas aeruginosa and Candida albicans)* was evaluated. The researchers observed that was a statistical difference in the Minimum Inhibitory Concentration (MIC) between the three extracts and that the Gram-positive

microorganisms and yeasts were those with greater sensitivity [34]. However, other scholars argue that the antimicrobial activity of propolis should be attributed to all its components, as identified that the APE action obtained from different locations was similar [35].

In this sense, the methods employed in the studies that evaluated the antimicrobial activity of propolis extract were very different, making it difficult to compare results. However, it is recognized that gram-positive bacteria are more susceptible to the action of various propolis extracts than the Gram-negative bacteria [36].

Despite the antibacterial action of propolis being well established in the literature, little is said of its antimicrobial action on yeasts. Thus, it was found that glycolic extract of propolis was effective on *C. albicans*, even in more diluted concentrations [37]. Researchers evaluated the in vitro effect of alcoholic propolis extract on 80 species of *Candida* yeasts and found that all strains were sensitive to this extract [38]. Comparing these two studies, it should be emphasized that in the first [37] it was used glycolic propolis extract, and not the alcoholic, used in most studies.

Using ethanol propolis extracts, the growth of *C. albicans* and S. aureus was not inhibited, demonstrating that the bioactive compounds of the samples did not have antibiotic activity [39]. By the results probably the propolis acted more as a physical barrier, than as an own biological protection for the micro-organisms tested [40].

Recently, researchers [41] obtained excellent results for *C. albicans*, especially for acetanolic fraction, wherein the minimum inhibitory concentration (MIC) is compared to the values found for the analyzed Gram-positive bacteria; contrary to the results found by other researchers [42], in which propolis extracts did not inhibit the growth of pathogens tested. The larger inhibition zone was of 4 mm at 20% and 30% concentrations, being not sufficient to reveal antibacterial activity.

Also in this sense, few studies have proposed the combination of propolis extract with antimicrobial agents, to decrease the clinical dose of certain antibiotics, as well as reducing the incidence of side effects and at the same time, enhance the antibiotical therapy [43-44].

Facing the exposed, the researches, overall, reveal more the antibacterial action of propolis than the antifungal.

Conclusion

Regarding the antifungal activity of red propolis, it was observed that its inhibitory process against *Candida* may have been influenced by the concentration of alcohol present in the extract, as there was evidence that the lowest concentration of propolis extract had higher antifungal record, compared to the higher concentration.

To test this hypothesis, it is suggested that research is performed with extracts of propolis and at the same time with alcohol, in both different concentrations and environmental conditions. So, this study offers subsidies for other professionals assess this fact, performing works with extracts prepared with different substances and concentrations, in order to test the antimicrobial action of these.

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