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Antifungal activity of commercial ethanolic and aqueous extracts of Brazilian propolis against Candida spp.

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ABSTRACT

Fourteen commercial ethanolic extracts (EEP) and five aqueous extracts (AEP) of Brazilian propolis were tested for antifungal activity *in vitro* by employing the agar diffusion assay to determine the susceptibility of *Candida* spp. Nystatin (100 IU), sterile distilled water and ethanol 93.2ºGL were used as positive, negative and solvent controls. Except for one sample, all EEP tested inhibited the *in vitro* growth of *Candida* spp., while the AEP extracts were only weakly inhibitory to the growth of these microorganisms. Four EEP products exhibited inhibition zones similar to those induced by nystatin. It was concluded that the commercial EEP extracts were more effective against *Candida* spp. than the aqueous extracts. This suggests that EEP could be used as an alternative medicine for the treatment of fungal infections of the oral cavity, such as oral candidosis (thrush) or denture stomatitis.

Keywords: ethanolic extract; aqueous extract; Brazilian propolis; *Candida* spp.

INTRODUCTION

Candidosis or thrush is by far the commonest oral fungal infection in humans. Caused by *Candida albicans*, it can be manifested in a variety of clinical presentations (Epstein et al., 1984). Other species of *Candida*, such as C. *tropicalis*, C. *krusei*, C. *parapsilosis* and C. *guilliermondi*, may also be found in the oral cavity but rarely cause disease (Budtz-Jorgensen, 1990). *Candida* spp. induce disease in the oral cavity by invading the tissues and inducing hypersensitivity (Samaranayake & Lamey, 1988).

Propolis is a resin used by bees to protect the hive against the invasion of microorganisms and is considered to be a natural antibiotic. It is used as a natural medicine in many parts of the world, but often randomly and without medical indication (Marcucci, 1995).

Several studies have demonstrated the biological versatility of propolis (Burdock, 1998; Park et al., 2002; Orsi et al., 2005), including an antimycotic effect (Ghaly et al., 1998; Ota et al., 2001; Sawaya et al., 2002; Martins et al., 2002). In Brazil, propolis extracts are readily available from commercial sources and they are used as a popular remedy for infectious conditions of the oral cavity and throat. Despite this widespread use by the community, the antimicrobial efficacy of the products available on the market is not documented.

The purpose of this article is to evaluate the antifungal activity against species of *Candida* of ethanolic and aqueous extracts of Brazilian propolis sold in Brazil.

MATERIAL AND METHODS

Samples of propolis extracts

Fourteen samples of ethanolic (EEP1-EEP14) and five of aqueous (AEP1-AEP5) extracts of Brazilian propolis (Table 1), commercially available from drugstores and shops specialized in apicultural products, were obtained in Belo Horizonte (MG, Brazil) and used in this study.

Microorganism strains and susceptibility test (agar diffusion test)

Strains of C. *albicans* (ATCC 18804), C. *tropicalis* (ATCC 750), C. *glabrata* (ATCC 2001), C. *parapsilosis* (ATCC 22019), C. krusei (ATCC 2340), and C. *guilliermondii* (ATCC 201935) were used as test organisms in this study. Each *Candida* spp. was incubated for 24h at 37ºC in Sabouraud dextrose broth (Biobrás-code 224-3-Brazil), and 0.1 mL of this culture (turbidity equivalent to a 5.0 McFarland standard) was spread on Sabouraud dextrose agar (Biobrás, Brazil). The agar disk diffusion susceptibility test was performed according to NCCLS guidelines (NCCLS, 1997). Tests were performed in triplicate.

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Table 1 - Ethanolic and aqueous Brazilian propolis extracts of different brands commercially available in drugstores in Belo Horizonte, Brazil.

Ethanolic Propolis (EEP)	Place of origin	% Propolis
EEP1	Ribeirão Preto-SP	10
EEP2	Santa Bárbara-MG	10
EEP3	Belo Horizonte-MG	10
EEP4	Belo Horizonte-MG	20
EEP5	Belo Horizonte-MG	20
EEP6	Ferros-MG	15
EEP7	Belo Horizonte-MG	20
EEP 8	São Paulo-SP	43
EEP 9	São Paulo-SP	35
EEP 10	Itapecerica- SP	10
EEP 11	Contagem-MG	10
EEP 12	Belo Horizonte-MG	10
EEP 13	Vespasiano-MG	20
EEP 14	Capão Redondo-SP	25
Aqueous Propolis (AEP)		
AEP1	São Paulo-SP	10
AEP2	Contagem-MG	15
AEP3	Capão Redondo-SP	20
AEP4	Vespasiano-MG	10
AEP5	Belo Horizonte-MG	10

Statistical analysis

The inhibitory zones of the various propolis solutions tested were compared by the nonparametric Kruskal-Wallis test. Differences were considered to be significant when $p<0.05$.

RESULTS

All *Candida* spp. tested in this study were susceptible to nystatin, moderately sensitive to ethanol, and insensitive to distilled water (Tables 2 and 3). C. *albicans* showed high sensitivity to EEP5, EEP6, and EEP10. These products were as active as nystatin. Intermediate inhibition zones were observed for EEP7, EEP8, and EEP11.

C. *glabrata* was highly sensitive to EEP6 and showed intermediate inhibition zones for EEP3 and EEP7. The inhibition zones observed for C. *glabrata* were significantly larger for EEP6 and nystatin than for any other EEP.

C. *tropicalis* demonstrated high sensitivity to EEP10-13 and intermediate sensitivity to EEP4, EEP5, and EEP7. The inhibition zones observed for EEP1-7 and EEP9 against C. *tropicalis* were significantly smaller than those seen for nystatin. However, EEP10-13 were as efficient as nystatin in inhibiting the in vitro growth of C. *tropicalis*. The values observed for AEP were significantly smaller than those observed for EEP.

C. *krusei* were susceptible to EEP4-6 and 8-11 and moderately sensitive to EEP12-13. None of the AEP samples inhibited C. *krusei* significantly.

C. *parapsilosis* showed sensibility to EEP8 and intermediate inhibition zones for EEP6 and EEP13. The AEP samples created inhibition zones significantly smaller than did nystatin.

C. *guilliermondii* was significantly less sensitive than other *Candida* spp. However, intermediate sensitivity was observed to EEP10 and EEP13.

EEP14 showed no inhibition zones against any species of *Candida*. There were no significant differences between the results observed for AEP brands and distilled water. Thus, the various AEP brands showed little or no effectiveness in the inhibition of the in vitro growth of the yeasts (Table 3). In general, the less aggressive pathogenic species, C. *parapsilosis*, C. *glabrata*, C. *krusei* and C. *guilliermondii*, were more sensitive than C. *albicans* to aqueous propolis extracts and nystatin.

DISCUSSION

Propolis contains approximately 55% resins and balsams, 30% wax, 10% oils and 5% pollen (Thompson, 1990). The chemical compounds in propolis have been isolated and analyzed in several different studies (Kujumgiev et al., 1999; Castaldo & Capasso, 2002; Kartal et al., 2003) and the flavonoid aglycones are recognized as possible factors responsible for its antibiotic activity (Bankova et al., 1995; Park et al., 2002; Santos et al., 2003). The chemical constitution of propolis varies with the type of bee, place of origin, extraction method and manufacturing quality control,

Table 2 - Average diameter and standard deviation (mm) of inhibition zones produced in agar diffusion tests against *Candida* species by 14 proprietary formulations of Brazilian propolis extracted in ethanol (EEP) and controls. Tests in triplicate (n=3).

EEP	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*C.parapsilosis*	*C. guilliermondi*
EEP1	10.6±0.57	15.5±0.70	13.5±0,50	9.3±0.57	12.0±1.00	12.0±1.00
EEP2	14.0±0.64 c	14.3±0.50	12.6±0.15	14.3±0.50	10.3±0.00	14.3±0.50
EEP3	14.6±0.50 c	15.0±1.00	16.0±0.64	14.3±1.00	13.3±0.05	14.3±0.57
EEP4	15.6±0.50 c	16.1±0.76	14.3±1.52	17.6±0.08	14.3±0.08	14.6±1.52
EEP5	20.0±0.64 a	16.1±0.76	14.3±1.52	17.6±0.08	14.3±0.08	14.6±0.52
EEP6	19.3±0.13 a	14.6±0.50	18.6±0.57 a	18.6±1.50	16.3±1.15	12.3±1.30
EEP7	16.6±0.15 b	16.0±1.00	16.0±1.00	15.6±0.50	15.6±1.52	13.0±0.64
EEP8	16.3±0.52 b	12.3±0.08	15.6±0.50	28.3±0.15	18.6±0.08	12.6±0.57
EEP9	12.6±0.15	15.3±1.52	14.3±0.50	26.6±1.08	12.6±0.30	11.6±0.50
EEP10	18.3±0.50 a	21.0±0.00	14.0±1.00	25.3±0.30	13.0±1.00	15.0±1.00
EEP11	16.0±0.60 b	19.0±0.00	10.3±0.08	17.6±0.08	12.6±0.08	11.6±1.15
EEP12	14.0±1.30 c	19.0±0.52	13.3±0.57	16.6±1.15	12.6±0.57	13.6±0.15
EEP13	12.3±0.57	18.6±1.15	14.6±1.15	16.6±0.15	16.0±1.73	15.0±.00
EEP14	0.0	0.0	0.0	0.0	0.0	0.0
Nystatin	21.0±0.60 a	20.3±1.52	22.0±0.64 a	20.3±0.50	27.0±1.00	26.0±0.15
Ethanol	8.0±1.00	9.0±0.50	8.0±0.00	7.0±1.0	8.0±0.00	9.0±1.00
Distilled water	0.0	0.0	0.0	0.0	0.0	0.0

Means within a column followed by the same letter do not differ (p>0.05), according to Kruskal-Wallis test.

Table 3 - Average diameter and standard deviation (mm) of inhibition zones produced in agar diffusion tests against *Candida* species by 5 proprietary formulations of Brazilian propolis extracted in water (AEP) and controls. Tests in triplicate (n=3).

AEP	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*C. parapsilosis*	*C. guilliermondii*
AEP-1	5.0±0.00 c	8.3±0.77	5.0±0.00	9.3±0.61	8.6±0.61	8.0±0.19
AEP-2	5.0±0.00 c	9.0±0.92	8.0±0.19	7.6±0.61	8.6±0.61	5.0±0.00
AEP-3	5.0±0.00 c	8.0±0.19	7.6±0.61	7.6±0.61	5.0±0.00	8.0±0.19
AEP-4	5.0±0.00 c	7.6±0.61	7.6±0.61	7.6±0.61	5.0±0.00	5.0±0.00
AEP-5	5.0±0.00 c	7.6±0.93	7.6±0.61	7.6±0.61	5.0±0.00	5.0±0.00
Nystatin	21.0±0.6 a	20.3±0.52	22.0±0.64	20.3±0.50	27.0±1.00	26.0±1.15
Ethanol	8.0±1.00 b	9.0±1.00	8.0±1.50	7.0±0.00	8.0±0.50	9.0±1.00
Distilled water	5.0±0.00 c	5.0±0.00	5.0±0.00	5.0±0.00	5.0±0.00	5.0±0.00

Means within a column followed by the same letter do not differ (p>0.05) according to Kruskal-Wallis test.

as can be seen from the variation in inhibition zones among the products tested in this study.

The fourteen commercial ethanolic extracts of propolis tested were from two Brazilian states, São Paulo and Minas Gerais. In this study, significant differences in inhibition zone diameter were observed among the propolis brands from São Paulo (EEP1, EEP8, EEP9, EEP10, EEP14) and Minas Gerais (EEP2, EEP3, EEP4, EEP5, EEP6, EEP7, EEP11, EEP12, EEP13). This suggests that there are differences in active compound concentrations between propolis samples of differing origin, as reported elsewhere (Kujumgiev et al., 1999; Marcucci et al., 2001; Banskota et al, 2001).

Several studies have demonstrated the antimycotic effect of Brazilian propolis (Banskota et al., 1998; Martins et al., 2002; Sahinler & Kaftanoglu, 2005), but no study has documented the antifungal effects of various commercial propolis solutions available in Brazil.

In the present study, some propolis extracts showed antifungal activity comparable to the positive control, nystatin. Nystatin is a polyene antifungal drug which has been standardized and accepted worldwide. The mean area of the inhibition zones produced by nystatin, for all *Candida* species, was similar to other reports (Ota et al., 2001; Martins et al., 2002). Despite its frequent use in the treatment of oral candidosis, nystatin has considerable side effects and resistant microorganisms have already been reported (Samaranayake & Holmstrup, 1989). Thus, the study of new antifungal drugs is of great relevance for the treatment of candidal infection.

The differences found in this study between

ethanolic and aqueous extracts seem to be associated with the loss of some essential active compounds from the propolis in aqueous extracts, such as flavonoids and prenylated cinnamic acids (Banskota et al., 1998; 2001). The EEP products, with higher antimicrobial activity, are the forms most frequently available in grocery stores, drugstores and specialized outlets for apicultural products.

The results of the present work indicate that several EEP effectively control the *in vitro* growth of *Candida* spp. and all EEP show greater antifungal effectiveness than the aqueous extracts. *In vivo* studies are needed for a better evaluation of the activity of propolis against oral candidosis in humans. EEP available commercially could be considered an alternative treatment for fungal infectious of the oral cavity, such as oral candidosis or denture stomatitis.

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RESUMO

Atividade antifúngica de extratos comerciais etanólicos e aquosos de própolis contra espécies de Candida

Quatorze extratos etanólicos comerciais de própolis (EEP) e cinco extratos aquosos (AP) foram testados *in vitro* para se determinar a susceptibilidade de espécies de *Candida*. O teste foi realizado utilizando-se o método de difusão em ágar. Nistatina (100 UI), água destilada estéril e etanol 93.2º GL foram usados como controles. Com exceção de uma amostra, todos os EEP inibiram o crescimento *in vitro* das espécies de *Candida*. AP foi fracamente efetivo na inibição do crescimento in vitro dos microorganismos testados. Quatro produtos EEP demonstraram zonas de inibição similares àquelas induzidas pela nistatina. Conclui-se que extratos etanólicos de própolis apresentam maior efetividade contra espécies de *Candida* quando comparados com o extrato aquoso. Este fato sugere que EEP pode ser considerado um possível medicamento alternativo para o tratamento de condições infecciosas fúngicas da cavidade oral, tais como candidíase e estomatite protética.

Palavras-chave: extrato etanólico; extrato aquoso; própolis; *Candida* spp.

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