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In vitro effect of *Paullinia cupana* (guaraná) on hydrophobicity, biofilm formation, and adhesion of *Candida albicans*' to polystyrene, composites, and buccal epithelial cells

Ermelinda Matsuura [a], *Janine Silva Ribeiro Godoy* [b], *Patrícia de Souza Bonfim-Mendonça* [b], *João Carlos Palazzo de Mello* [c], *Terezinha Inez Estivalet Svidzinski* [b], *André Gasparetto* [a], *Sandra Mara Maciel* [a,*]

[a] *Department of Dentistry, State University of Maringá, Av. Mandacaru 1550, CEP 87080-000, Maringá, PR, Brazil*
[b] *Department of Clinical Analysis, State University of Maringá, Av. Colombo, 5790, bloco T20 sala 203, CEP 87020-900, Maringá, PR, Brazil*
[c] *Departament of Pharmacy and Pharmacology, State University of Maringá, Av. Colombo, 5790, bloco K80, CEP 87020-900, Maringá, PR, Brazil*

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ABSTRACT

Objective: *In vitro* evaluation of the effect of guaraná (GUAR) on cell surface hydrophobicity (CSH), on biofilm formation, and on adhesion of *C. albicans* to polystyrene, to composite resins, and to buccal epithelial cells (BEC).

Materials and methods: Lyophilised aqueous extract of GUAR was tested on *C. albicans* ATCC (90028). The effect of GUAR was evaluated by examining the CSH of *C. albicans*, as determined by microbial adhesion to hydrocarbons test, by assessing biofilm production and through adhesion assays (microplates of polystyrene, BEC and composites). One nanoparticle (Z350®) and two microhybrid (LLis®, Opallis®) composites were tested. Scanning electron microscopy (SEM) was used to analyse adhesion of *C. albicans* composites. Assays were performed in triplicate and the results analysed by Chi-square test, Kruskal–Wallis test and Dunn's Multiple Comparison *post hoc* test at 5% significance level.

Results: GUAR did not inhibit growth of *C. albicans* at any concentration, but it reduced adhesion to polystyrene surface ($p < 0.001$). Exposure to GUAR did not change CSH and biofilm formation, but it increased adhesion of *C. albicans* to the nanoparticle composite ($p = 0.042$) and reduced its adhesion to BEC ($p < 0.001$). SEM confirmed an aggregatory pattern of adhesion of *C. albicans* to composites.

* *Corresponding author at*: Universidade Estadual de Maringá, Av. Mandacaru 1.550, CEP 87080-000, Maringá, PR, Brazil. Tel.: +55 43 91165306; fax: +55 44 30319051.
E-mail addresses: ematsuura1@gmail.com (E. Matsuura), sandramaciel53@gmail.com (S.M. Maciel).

Abbreviations: BEC, buccal epithelial cells; CFU, colony forming units; CHLOR, chlorhexidine; CSH, cell surface hydophobicity; GUAR, guaraná (*Paullinia cupana*); INOC, inoculum; MIC, minimum inhibitory concentration; OD, optical density; PBS, phosphate buffered saline; SDA, Sabouraud's dextrose agar; TB, test-bodies.

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Conclusion: GUAR increased the adhesion of *C. albicans* to the surface of the nanoparticle composite. However, it reduced the adhesion of *C. albicans* to BEC and to polystyrene, which reveals its potential use in prevention of oral diseases.

1. Introduction

Candida is a commensal microorganism present in normal human microbiota,[1–4] considered an opportunistic agent as under certain circumstances it can become pathogenic, causing approximately 80% of all fungal infections.[3–6] These infections can range from superficial lesions to critical and invasive systemic disseminations, with mortality rate between 40% and 60%.[7]

Candida albicans is the most prevalent of the *Candida* species in human oral cavity.[1,5,6,8,9] The capacity of this yeast to adhere to any oral substrate, the first and essential stage in biofilm formation, is one of the main reasons for its pathogenic character.[10,11] An important predisposing factor to infections[16] is that *C. albicans* can adhere both to biotic surfaces, such as teeth or mucosa,[1,12] and to abiotic surfaces, such as acrylic denture base,[1–3,5,8,9,13] orthodontic metal braces,[14] and surfaces of dental restorations.[10,15] Cell surface hydrophobicity (CSH) contributes to the ability of *C. albicans* to adhere to inert surfaces.[13,17,18] Stomatitis caused by *C. albicans* affects about 67% of elderly denture wearers with poor oral hygiene,[1,10,13,19] which may explain why a considerable body of research focuses on the adhesion of the yeast to prosthetic materials based on acrylic resin.[1–3,5,8,9,13] However, yeast infections and their sequelae have increasingly been found in groups other than prostheses wearers, particularly patients under immunosuppressive conditions.[4,10] Direct restorative materials should also be assessed as potential adhesion surfaces and reservoirs of *C. albicans* in the oral cavity.[10] Nevertheless, few studies have examined the adhesion of this yeast to composite resins.[10,15,20,21]

The use of oral chlorhexidine (CHLOR), despite its known benefits, should be carefully monitored because of its side effects.[22] Research on natural products has increased in recent years due to the search for more affordable substances with improved pharmacological activity and lower toxicity.[23–25] Several herbal extracts have been tested in Dentistry.[21,25]

There are several native Brazilian plants potentially medicinal,[23,25] although many of them have not been scientific validated.[23] *Paullinia cupana*, known as guaraná (GUAR), is a native plant of central Amazon. The seed extract is used in the preparation of stimulating beverages, as it is rich in caffeine and also contains flavonoids (catechins and epicatechins) and tannins.[26] Both *in vitro* and *in vivo* studies show different properties of GUAR, such as antioxidants, anti-amnesic, stimulant, adaptogenic, antidepressant, anti-stress,[27,28] and antimicrobial.[25] Studies on its antimicrobial property are of great interest to Medical Sciences in general and to Dentistry in particular.

The aim of this study was to evaluate *in vitro* effect of GUAR extract on CSH, on biofilm formation, and on adhesion of *C. albicans* to buccal epithelial cells (BEC), to polystyrene surfaces, and to composite resins.

2. Materials and methods

The study was approved by the Ethics Committee in Research Involving Human Participants (Protocol #408/2009) and all procedures were performed in accordance with Resolution196/96 CNS.

2.1. *C. albicans* strain, GUAR extract and controls

C. albicans (ATCC 90028) was obtained from the Medical Mycology Laboratory of the State University of Maringá (Maringá, PR, Brazil). It was reactivated 24 h before each experiment in YPD (1% yeast extract, 2% peptone and 2% dextrose, Difco, USA) dissolved in distilled water at pH 6.5. It was then cultivated for 24 h in YPD agar (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) and resuspended in phosphate buffered saline (PBS, 2.7 mM KCl and 157 mM NaCl, in 10 mM of potassium phosphate buffer, pH 7.2). The concentration of the inoculum (INOC) was adjusted using a Neubauer haemocytometer (Sigma, USA).

Seeds of *Paullinia cupana* were obtained in the region of Alta Floresta city (MT, Brazil). The crude extract was prepared with acetone/water (70:30, v/v) using Ultra-Turrax UTC115KT (IKA Works, Wilmington, NC, USA). Organic solvent was eliminated on a rotatory evaporator under reduced pressure (Rotavapor, R-200, Büchi, Switzerland) and the material was lyophilised to yield the crude extract (EBPC; patent pending PI0006638-9). EBPC was partitioned with ethyl acetate, which resulted in an ethyl-acetate fraction and an aqueous fraction,[28,29] the latter being controlled according to Klein et al.[30] The aqueous fraction powder was suspended in ultrapure sterile distilled water to a final concentration of 10 mg/mL.

A solution of CHLOR 0.125% (chlorhexidine gluconate 2%, FGM, Brazil) was used as positive control and PBS as negative control.

2.2. Minimum inhibitory concentration (MIC) determination

MIC was determined by using broth microdilution method in accordance with the M27-A3 document of the Clinical and Laboratory Standards Institute,[31] with some adjustment for natural products. GUAR was 10-fold diluted from a 10 mg/ml concentration and compared to INOC 5×10^5 colony forming units per millilitre (CFU/ml). Control CHLOR was diluted from a 2% concentration.

2.3. Cell surface hydrophobicity (CSH)

CSH was determined according to microbial adhesion to hydrocarbons[32,33] with the following modifications: either GUAR (350 μL, 10 mg/mL), CHLOR (0.125%) or PBS were added