Electrophoretic profile of cell wall extracts from *Candida albicans* samples isolated from women with vulvovaginitis

Perfil eletroforético de extratos da parede celular de amostras de *Candida albicans* isoladas de mulheres com vulvovaginite

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Jessica Dayana Batista
Farmacêutica pela Universidade Estadual do Oeste do Paraná
Instituição: Hospital Universitário do Oeste do Paraná, Universidade Estadual do Oeste do Paraná – UNIOESTE
Endereço: Avenida Tancredo Neves, 3224, Cascavel, Paraná CEP: 85806-470, Brasil
E-mail: rinaldogandra@unioeste.br

Jessica Vieira
Especialista em Acupuntura, Mestranda em Ciências Farmacêuticas pela Universidade Estadual do Oeste do Paraná
Instituição: Hospital Universitário do Oeste do Paraná, Universidade Estadual do Oeste do Paraná – UNIOESTE
Endereço: Avenida Tancredo Neves, 3224, Cascavel, Paraná CEP: 85806-470, Brasil
E-mail: jessica.vieira93@hotmail.com

Eloiza Cristina Martelli
Especialista em Farmacologia e Interações Medicamentosas, Mestranda em Ciências Farmacêuticas pela Universidade Estadual do Oeste do Paraná
Instituição: Hospital Universitário do Oeste do Paraná, Universidade Estadual do Oeste do Paraná – UNIOESTE
Endereço: Avenida Tancredo Neves, 3224, Cascavel, Paraná CEP: 85806-470, Brasil
E-mail: eloiza.martelli@hotmail.com

Jessica Cassia da Silva
Especialista em Análises Clínicas, Mestre em Ciências Farmacêuticas pela Universidade Estadual do Oeste do Paraná
Instituição: Hospital Universitário do Oeste do Paraná, Universidade Estadual do Oeste do Paraná – UNIOESTE
Endereço: Avenida Tancredo Neves, 3224, Cascavel, Paraná CEP: 85806-470, Brasil
E-mail: jessica.cassias@gmail.com

Rafaela de Souza Marquezoni
Farmacêutica pela Universidade Paranaense
Instituição: Hospital Universitário do Oeste do Paraná, Universidade Estadual do Oeste do Paraná – UNIOESTE
Endereço: Avenida Tancredo Neves, 3224, Cascavel, Paraná CEP: 85806-470, Brasil
E-mail: rinaldogandra@unioeste.br

Maria Gabriele Cerny
Estudante de Farmácia pela Universidade Estadual do Oeste do Paraná
Instituição: Hospital Universitário do Oeste do Paraná, Universidade Estadual do Oeste do Paraná – UNIOESTE
Endereço: Avenida Tancredo Neves, 3224, Cascavel, Paraná CEP: 85806-470, Brasil
E-mail: rinaldogandra@unioeste.br

Rinaldo Ferreira Gandra
Doutorado em Microbiologia pelo Instituto de Ciências Biomédicas da Universidade de São Paulo
Instituição: Hospital Universitário do Oeste do Paraná, Universidade Estadual do Oeste do Paraná – UNIOESTE
Endereço: Avenida Tancredo Neves, 3224, Cascavel, Paraná CEP: 85806-470, Brasil
E-mail: rinaldogandra@unioeste.br

ABSTRACT
Candida albicans is a common commensal fungus in the human microbiota. It causes not only opportunistic infections, as in vulvovaginal candidiasis, but also allergic reactions in people sensitized to the fungus. The present study evaluated the ability of Coca liquid to extract glycoproteins considered fungal antigens from *Candida albicans* samples, with protein bands being visualized by SDS-PAGE electrophoresis. Five strains of *Candida albicans* were used, which were subjected to extraction with Coca liquid (0.28% NAHCO3, 0.49% NaCl), protein and carbohydrate were dosed in the supernatant of the extract and subsequently submitted to electrophoresis. We concluded that the Coca Liquid had great capacity for protein extraction, in addition to being a simple and inexpensive method.

Keywords: protein, coca liquid, sds-page.

RESUMO
Candida albicans é um fungo comensal comum na microbiota dos seres humanos. Provoca não só infecções oportunistas, como na candidíase vulvovaginal, mas também reações alérgicas em pessoas sensibilizadas pelo fungo. O presente estudo avaliou a capacidade do líquido de Coca em extrair glicoproteínas consideradas antígenos fúngicos, de amostras de *Candida albicans*, sendo visualizadas as bandas proteicas através de eletroforese SDS-PAGE. Foram utilizadas 5 cepas de *Candida albicans* que foram submetidas à extração com o Líquido de Coca (0,28% $NAHCO_3$, 0,49% NaCl), no sobrenadante do extrato foram realizadas as dosagens de proteínas e carboidratos e posteriormente submetidos à eletroforese. Concluímos que o Líquido de Coca apresentou uma ótima capacidade de extração de proteínas, além de ser um método simples e barato.

Palavras-chave: proteína, líquido de coca, sds-page.

1 INTRODUCTION

Candida albicans is a common commensal fungus in the human microbiota, and can colonize several body sites, such as oropharynx, buccal cavity, skin folds, bronchial secretions, vaginal mucosa, urine, feces, among others (Rocha et al., 2020). However, in

immunocompromised hosts, these yeasts can act as pathogens. Many pathological processes can facilitate the colonization and subsequent infection of the host by Candida albicans. Among these, vulvovaginal candidiasis (Andrioli et al., 2009).

The cell wall of fungi is formed by approximately 80 to 90% of carbohydrates, 6 to 25% of proteins and a small portion of lipids (1 to 7%) (Lopes-ribot et al., 1991). One of the virulence factors and also considered fungal antigens and allergens of *C. albicans* are proteins, which mediate adherence and invasion to the target tissue and induce immediate and delayed hypersensitivity responses in the host (Fernandes, 2008).

Antigenic determinants have been the subject of several studies that demonstrate their existence in the cell wall, cytoplasm and metabolic compounds. However, cell wall antigens are the most studied, due to their ease of extraction (Del Negro, 1993).

The association of polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE) has allowed a more accurate study of the antigenic characteristics of materials extracted from the cell wall, in addition to determining the molecular weight of antigenic fractions (Del Negro, 1993). The present work aimed to evaluate the ability of Coca liquid to extract glycoproteins considered fungal antigens from *Candida albicans* samples and to trace the electrophoretic profile of the strains.

2 MATERIALS AND METHODS

Five strains of *Candida albicans* from the Culture Collection of the Mycology Laboratory of the Universidade Estadual do Oeste do Paraná were used in this study. In Erlenmeyer flasks containing 200 mL of Sabouraud broth, 10^5 CFU/mL of yeasts from cultures on Sabouraud Agar were added for 48 h at 36-37°C. These flasks were incubated for 5 days at 36-37°C. After the incubation period, the cells were separated from the culture medium by centrifugation at 4,000 rpm for 5 minutes. Then they were washed three times with sterile distilled water and placed in an oven at approximately 40°C for 24 hours for dehydration. The cells, thus dried and weighed, were placed in contact with Coca Liquid (0.28% NAHCO3, 0.49% NaCl), at a concentration of 5% w/v and kept at 4°C for one week. Then the samples were centrifuged, and the carbohydrates and proteins were dosed in the supernatant using the Antrona and Bradford method, respectively. Subsequently, the supernatants were dialyzed, lyophilized and stored at -20ºC. The lyophilized samples were resuspended in 500µL of sterile distilled water and subjected to fractionation, according to their molecular weight, by electrophoresis in polyacrylamide gel plus sodium dodecyl sulfate (SDS-PAGE). After the electrophoretic run, the gels were stained with Cromassie Blue where they remained for 12

hours. The discoloration was carried out by bleaching solution, until the protein bands were visible.

3 RESULTS AND DISCUSSION

With the incubation in a culture medium of 10^5 CFU/mL of yeast, a weight of dry cell extract was obtained for the 5 samples ranging from 0.675 to 0.912 g. The amount of Coca liquid added corresponded to the weight of the dry extract obtained according to the proportion 5% w/v.

The concentration of proteins and carbohydrates obtained from the extraction with the Coca Liquid varied from one strain to the other, demonstrating the method's extraction capacity as well as the biochemical diversity existing among the *Candida albicans* samples (Table 1). The highest protein concentration was obtained by strain 5 (35.8mg/mL) and the highest carbohydrate concentration was obtained with strain 2 (882.4mg/mL).

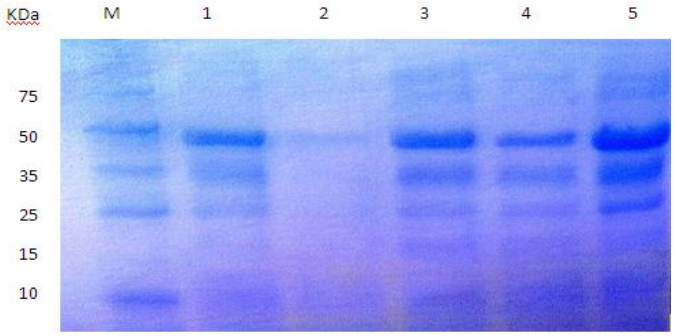
According to Gandra *et al.* (2001), when fungal cells come into contact with Coca liquid at 4°C for a week, protein extraction is slow, but it promotes a high yield of cell wall components.

Table 1- Protein and carbohydrate concentration of *Candida albicans* cell wall extracts obtained with Coca liquid.

C*andida albicans* sample	**Protein concentration (mg/mL)**	**Carbohydrate concentration (mg/mL)**
1	21,7	272,4
2	6,6	882,4
3	28,5	148,4
4	12,4	344,4
5	35,8	18,4

Figure 1 shows the electrophoretic profile of the glycoproteins of the 5 strains of *Candida albicans* obtained from the extraction with Coca liquid. Samples 1, 2, 3, 4 and 5 showed respectively 4, 4, 6, 6 and 6 protein fractions, and fractions with molecular weight of approximately 50, 35, 25 and 10 KDa are common among the five types of extracts.

Figure 1 – Electrophoretic profile SDS-PAGE of 5 samples of *Candida albicans* extracted with Coca liquid. M: Marker.



Rosa *et al* (2000), using a range of similar molecular masses, observed that bands with masses greater than 45 kDa are repeated in most species, suggesting that they may be representative of the genus.

In the study by Rodrigues (2001), polyacrylamide gel electrophoresis was performed on protein extracts from 14 randomly chosen strains of *C. albicans*, which allowed the detection of profiles containing approximately 15 - 20 electrophoretic bands. The electrophoretic profiles of the strains were very similar to each other.

4 CONCLUSIONS

The Coca liquid showed high extraction capacity for proteins and carbohydrates from *Candida albicans*, in addition to being a quick, simple and inexpensive procedure.

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