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ORIGINAL ARTICLE

Effectiveness of five antidandruff cosmetic formulations against planktonic cells and biofilms of dermatophytes

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Abstract This study aimed to investigate the *in vitro* antifungal effectiveness of five different formulations against dandruff and ringworm dermatophytes. *Candida albicans* was also included in our assays. Fungal susceptibility tests were performed with planktonic cells and biofilms of reference strains. Microbiological and physicochemical quality parameters were assessed for all formulations. Our data indicated that the formulations were effective against the dermatophytes strains, and to our knowledge, the effectiveness of cosmetic formulations against fungal biofilms is shown for the first time. The formulations were considered effective against the explored dermatophytes and were considered safe given the adequate microbiological and physicochemical characteristics shown in the proposed assays.

1. Introduction

Dermatophytes are a group of fungi that have the capacity to invade keratinized tissues of humans and animals such as skin and nails. The infection is generally limited to the *stratum corneum* due to poor or no penetration of fungi on deeper tissues layers of immunocompetent individuals. Specifically, the skin of the scalp is thick and contains numerous sebaceous glands, and as a result of the high density of hair follicles and sebum production, it is susceptible to pathological fungal infections, inflammatory diseases and formation of sebaceous cysts. Common conditions in this context

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include dandruff and ringworm (Gupta and Nicol, 2004; Turner et al., 2012).

Dandruff is a common scalp complaint generally characterized by itching and presence of flakes on the skin and hair of the scalp. The severity can range from mild to severe, with higher prevalence and severity in men, due to factors that include testosterone metabolism to 5-α-dihydrotestosterone, which is more potent than testosterone to trigger sebum production. Dandruff pathophysiology is still not completely understood, but symptoms can be brought on by changes in weather and air humidity, scratching and emotional stress. Accelerated proliferation of epidermal cells combined to increased exfoliation of keratinizing epidermal cells can lead to the breakdown of the columnar structure of the *stratum corneum* in the scalp, with increased formation of cell aggregates (flakes), and the role of *Malassezia furfur* has been discussed as an important factor regarding the evolution of the disease and treatment (Kerr et al., 2011; Gaitanis et al., 2012; Dessinioti and Katsambas, 2013).

Ringworm or *Tinea capitis* is an exogenous infection of the skin and hair of the scalp caused by dermatophytes of the genera *Trichophyton* and *Microsporum*, with wide distribution in tropical and subtropical countries, although the prevalence is variable due to factors such as the weather and hygiene conditions, host immune system, fungal virulence and treatment options available. The disease most frequently affects children under 10 years old, preschool and school aged children, and may affect postmenopausal women and immunocompromised patients, being *Trichophyton rubrum* and *Trichophyton mentagrophytes* the most common species detected in Brazilian patients (Aquino et al., 2007; Godoy-Martinez et al., 2009; Costa-Orlandi et al., 2014).

Different cosmetic formulations are available to manage fungal infections and inflammatory diseases of the scalp worldwide without need of medical prescription, being generally safe and effective options in eradicating or providing relief of the main symptoms. In general, cosmetic and pharmaceutical products for treating dandruff and ringworm include at least one the following type of active ingredients: keratolytics (sulfur, salicylic acid), antimicrobial natural products (*Melaleuca* sp. oil, *Aloe vera*), regulators of sebum production (zinc), and antifungal drugs (selenium sulfide, azole drugs) (Kumar and Mali, 2010; Dessinioti and Katsambas, 2013). Conversely, few studies have demonstrated the quality of such formulations in controlled *in vitro* experiments. Most of the data are kept with the manufacturer for patent or product registration. Poor evidence is available for scientific purposes, and reports of sales of ineffective formulations are not new worldwide (Wong et al., 2000; Lundov et al., 2009).

Therefore, this study aimed to investigate the antifungal effectiveness of five different cosmetic formulations widely sold in Brazil against ringworm and dandruff dermatophytes *in vitro*. Moreover, microbiological and physicochemical quality parameters were assessed for all formulations. Our data indicated that the formulations can be considered safe given the adequate microbiological and physicochemical characteristics shown in the proposed assays, and were effective against the tested dermatophytes. To our knowledge, the effectiveness of Brazilian cosmetic formulations against fungal biofilms is shown for the first time. Given the scarcity of studies in this field, our data become even more relevant.

2. Materials and methods

2.1. Samples

The products assessed in this study are manufactured and widely sold in Brazil, and are described in Table 1 with the respective composition available at the container box and label of the products. All items were acquired at a local market, and shelf conditions like sun and humidity exposure were verified for selecting each sample. Primary package quality was assessed by visual inspection: the product should not present fissures, broken seal, poor visibility of label information and other quality deviations. The selected items were from the same production registration number (batch code), with shelf life of safe use until 2017.

2.2. Physicochemical analysis

Color, odor and formulation aspect were assessed visually. Viscosity data were recorded using a Brookfield viscosimeter operated at 25 °C, and pH was assessed by direct reading using a pHmeter (Gehaka).

2.3. Viable microbial counting

Total viable bacterial count was determined as described by Shaqra and Al-Groom (2012), with slight modifications. For each formulation, 1 mL of the preparation was dispersed in sterile PBS containing 0.5% polysorbate 80 (preservative neutralizer) and 10-fold serial dilutions were made under aseptic conditions. Spread plate technique was performed on a 200 μL aliquot taken from the appropriate dilution using Manitol agar (Difco) for *Staphylococcus aureus*, Cetrimide agar (Difco) for *Pseudomonas* gender, MacConkey agar (Difco) for Gram-negative organisms (mainly coliforms), and Sabouraud dextrose agar (Oxoid) for yeasts and molds. All agar plates were prepared with 0.5% polysorbate 80.

Results were considered positive if fungal growth appeared on the inoculated plates after incubation at 28 °C for 3–7 days, and if bacterial growth appeared after overnight incubation at 37 °C. *Candida albicans* was assessed as described for bacteria, but Sabouraud dextrose agar was used with incubation at 28 °C. Standard (ATCC) strains were used as positive controls for each media (Table 2), and plates with sterile agar were used as negative controls.

2.4. Challenge test of the preservative system

This assay was performed as described in the United States Pharmacopeia (USP, 2014), in duplicate for each product. Stock cultures of reference microorganisms (Table 2) was grown in adequate liquid media, harvested by centrifugation (3000*g*, 15 min, 10 °C), washed with sterile saline solution and resuspended in sterile fresh liquid media to obtain a microbial count of about 1×10^8 CFU/mL, determined by turbidimetric measurements. The test was conducted in sterile falcon tubes in which sufficient volume of each product could be transferred. Each tube was inoculated with 1% in volume of each standardized inoculum and mixed. Microorganisms were added to the products such that the final concentration of the