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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.

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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 μ L 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 (3000g, 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

Table 1 Composition of the Formulations used in the tests.

Formulation	Active ingredient(s)	Raw material composition (INCI name nomenclature)
Biocilin antidandruff conditioner	Piroctone olamine 0.006%	Water, Cetearyl Alcohol, Mineral Oil, Cetrimonium Chloride, Glyceryl Stearate, Stearamidopropyl Dimethylamine, Cyclomethicone, Amino Bispropyl Dimethicone, Fragrance, Phenoxyethanol, Citric Acid, Dimethicone, Behentrimonium Methosulfate, Trideceth-12, Pentaerythrityl Tetra-Di-T-Butyl Hydroxyhydrocinnamate, TEA-Dodecylbenzenesulfonate, Benzyl Salicylate, Hexyl Cinnamal, Linalool, <i>Argania spinosa</i> Kernel Oil, Hydrolyzed Keratin, Methylchloroisothiazolinone, Methylisothiazolinone, Tetrasodium EDTA
Biocilin antidandruff shampoo	Piroctone olamine 0.2%	Water, Cocamidopropyl Betaine, Sodium Laureth Sulfate, Glycol Distearate (and) Sodium Laureth Sulfate (and) Cocamidopropyl Betaine, Cocamide DEA, Ammonium Lauryl Sulfate, Laureth-2, Propylene Glycol, Amodimethicone (and) Trideceth-12 (and) Cetrimonium Chloride, Parfum, Guar Hydroxypropyltrimonium Chloride, Menthol, Citric Acid, Hydrolyzed Keratin, PEG-120 Methyl Glucose Dioleate, Methylisothiazolinone (and) Methylchloroisothiazolinone, Disodium EDTA, <i>Argania spinosa</i> Kernel Oil, Benzyl Salicylate, Hexyl Cinnamal, Linalool
Biocilin antidandruff gold shampoo	Zinc pyrithione 2% and Salicylic Acid 0.5%	Water, Sodium Laureth Sulfate, Cocamidopropyl Betaine, Cocamide DEA, Zinc Pyrithione, Triethanolamine, Laureth-2, Propylene Glycol, Acrylates/C10–30 Alkyl Acrylate Crosspolymer, Salicylic Acid, Phenoxyethanol, Fragrance (Parfum), Panthenol, Guar Hydroxypropyltrimonium Chloride, Hydrolyzed Lupine Protein, Amodimethicone, Menthol, <i>Aloe barbadensis</i> Leaf Extract, <i>Arctium majus</i> Root Extract, Benzyl Salicylate, Hexyl Cinnamal, Linalool, Trideceth-12, Cetrimonium Chloride, Citric Acid
Biocilin antidandruff hair tonic	Piroctone olamine 0.3%	Water, Alcohol, Propylene Glycol, Piroctone Olamine, Phenoxyethanol, PEG-40 Castor Oil, Fragrance (Parfum), Citric Acid, Menthol, Benzyl Salicylate, Hexyl Cinnamal, <i>Aloe barbadensis</i> Leaf Extract, <i>Arctium majus</i> Root Extract, Linalool, Limonene, Butylphenyl Methylpropional, Geraniol, Citronellol, Methylchloroisothiazolinone, Methylisothiazolinone, Tetrasodium EDTA
Biocilin antidandruff gold hair tonic	Piroctone olamine 0.3%	Water, Alcohol, Propylene Glycol, Hydrolyzed Lupine Protein, Piroctone Olamine, Citric Acid, Phenoxyethanol, PEG-40 Castor Oil, Methylisothiazolinone (and) Methylchloroisothiazolinone, Menthol, Benzyl Salicylate, Hexyl Cinnamal, Linalool, D-Limonene, Butylphenyl Methylpropional, Geraniol, Citronellol, Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde

inoculum ranged from 1×10^5 to 1×10^6 CFU/mL of formulation. Reference parameters for category 2 products were used for assessing the results: for bacteria, not less than 2log reduction from the initial calculated count at 14 days, and no increase from the 14 days' count at 28 days. For yeasts and molds, results should have no increase from the initial calculated count at 14 and 28 days.

2.5. Antifungal activity against planktonic cells

The antifungal activity of each formulation was determined by the inhibition zone assay described by Schmidt-Rose et al.

Table 2 Microorganisms used in challenge test (USP, 2014).

Micro-organisms	
Bacteria	<i>Escherichia coli</i> ATCC 8739 <i>Pseudomonas aeruginosa</i> ATCC 9027 <i>Staphylococcus aureus</i> ATCC 6538
Yeast	<i>Candida albicans</i> ATCC 10321
Filamentous fungi	<i>Aspergillus niger</i> ATCC 16404

(2011), with some modifications. The chosen strains (Table 3) are well known pathogens involved in dandruff, ringworm and other dermatophytosis infections that should be controlled with the active molecules of the cosmetic formulations. Briefly, homogeneous suspensions of the dermatophytes stock cultures were prepared with recent cultures in phosphate buffered saline (PBS) using the McFarland 1 scale for turbidity adjustment ($\sim 3 \times 10^8$ CFU/mL), and 100 μ L of the suspension was distributed through spread plate method in Sabouraud dextrose agar (Oxoid) (*Candida* and *Trichophyton* ATCC strains), and in Sabouraud agar plates supplemented with 1% (v/v) of pure olive oil (*M. furfur* strain). Both agar preparations included 0.5% polysorbate 80 (preservative neutralizer).

After allowing the suspension to dry, two holes were made in each plate with the help of a sterile glass punch with

Table 3 Microorganisms used in antifungal assays.

Micro-organisms	
Filamentous fungi	<i>Trichophyton mentagrophytes</i> ATCC 9533 <i>Trichophyton rubrum</i> ATCC 28189
Yeasts	<i>Candida albicans</i> ATCC 10321 <i>Malassezia furfur</i> ATCC 14521

diameter of 5 mm, and 50 μL of a 50% suspension of each formulation (prepared in PBS with 0.5% polysorbate 80) was introduced in each hole. Following overnight incubation of the plates for *C. albicans* and 3–7 days for other genders at 28 °C, inhibition of fungal growth was determined by measurement of the inhibition zone area. For each formulation, duplicates were tested in four independent experiments. PBS prepared with 0.5% polysorbate 80 was used as a control.

2.6. Biofilm formation assays

Fungal biofilms were prepared in 96 wells non-treated polystyrene plates to test the efficacy of the formulations. The micro-organisms used are listed in Table 3. For *M. furfur*, biofilm formation was performed as previously described (Rukayadi and Hwang, 2007), with slight modifications. The ATCC strain was grown on Sabouraud Dextrose Broth or Agar (Oxoid) supplemented with 1% (v/v) of pure olive oil, following incubation at 35 °C for 5 days. The cells were then transferred to a Sabouraud supplemented broth and incubated in an orbital shaker at 35 °C, 150 RPM, for 3 days. The cells were centrifuged, the supernatant was removed, and the pellet was washed with sterile 0.9% saline. Sabouraud supplemented broth was added to reach an adjusted concentration of 1×10^6 CFU/mL. A total of 200 μL of the suspension was transferred to 96 well flat bottom microtiter plates. The plates were incubated for 24 h at 35 °C at 70 RPM to allow biofilm adhesion. After, the supernatants were aspirated and each well was washed three times with sterile saline and left to air dry.

Biofilm formation of *Trichophyton* species was performed as described by Costa-Orlandi et al. (2014), with some modifications. The ATCC strains of *T. rubrum* and *T. mentagrophytes* were grown on potato dextrose agar (Difco) and incubated at 28 °C for 5 days or until sporulation was seen. The inoculum was prepared by covering the cultures with 5 mL of sterile saline, which was adjusted to a final concentration of 1×10^6 CFU/mL. Then, 300 μL of inoculum was added to 96 well flat bottom plates. The plates were then incubated without agitation at 37 °C for 3 h for biofilm pre-adhesion. After this time, the supernatant was removed and 300 μL of RPMI 1640 medium was added to each well. The plates were incubated at 37 °C for 72 h. After, the biofilms were washed three times with sterile saline and left to air dry.

For *C. albicans*, biofilm formation was performed as described by Kagan et al. (2014), with modifications. *C. albicans* was grown in Sabouraud Agar medium and after in Sabouraud broth. The culture was incubated overnight in an orbital shaker at 28 °C, 200 RPM. Following, cells were centrifuged, washed three times in sterile saline, and resuspended in fresh dextrose Sabouraud broth. Biofilms were formed in 96 well plates by adding 300 μL of the 1×10^6 CFU/mL standardized cell suspensions. Plates were incubated at 37 °C overnight, and after, the biofilms were washed three times with sterile saline and left to air dry.

2.7. Biofilm eradication assays

For assessing the antibiofilm effects of the formulations, we employed the method of Dias-Souza et al. (2013) with modifications. The dry biofilms prepared in the previous section were used briefly after washing. Aliquots of 300 μL of each 50%

suspension prepared for each formulation (in PBS with 0.5% polysorbate 80) were then dispensed in each well. The plates were again incubated for 24 h in the conditions described. After this period, wells were gently aspirated and washed as described. Viability staining tests were performed using 50 μL of a 1% MTT solution. Pink (to purple) color indicated fungal growth, and yellow color indicated effective activity against fungal biofilms (biofilm eradication). Three independent experiments were performed for each fungal biofilm using four wells for each formulation.

2.8. Statistical analysis

Homoscedasticity and normality were assessed through Bartlett's test and through Shapiro–Wilk test, respectively. Mean diameters of the inhibition zones were analyzed using ANOVA followed by Tukey test. The significance level was set at $p < 0.05$, and highly significant values were set as $p < 0.001$. All analyses were carried out in Minitab 17 statistical package for Windows.

3. Results and discussion

3.1. Physicochemical analysis

Physicochemical parameters play an important role in defining and controlling many attributes such as shelf life stability, ease of flow on removal from primary packaging, spreading on application to hair, product consistency in the package and product esthetics for users. The results of color, odor and formulation aspect were compared to the information described in the label. Viscosity and pH were analyzed and when possible compared to the legislation or with specific information gathered from the manufacturer quality report, which were used as reference values. All parameters were adequate with the legislation and with the manufacturer standards (Table 4).

3.2. Viable microbial counting and challenge test of the preservative system

The formulations were found to be free of microbial contamination and supported the contamination. Total viable bacterial counts for the five formulations were negative and remained unchanged during the study. CFU counts for the challenge test contamination, which uses specific bacteria and fungi that can lead to spoilage of cosmetic products, were also negative for all micro-organisms in all testing periods. Therefore, the preservative system was considered effective. Like in developed countries where the prevalence of contamination cosmetics available for sale is rare (Campana et al., 2006), considering the adopted parameters of pharmaceutical development and industrial manufacturing, the tested products responded to these tests adequately.

There are few studies that have described the microbial quality of cosmetic products. A recent research conducted in Jordan showed that from a total of 57 brands of hair care products, 18.5% of them were found to be contaminated with high microbial populations. In Nigeria, Okeke and Lamikanra (2001) investigated the microbiological quality of 49 skin-moisturizing creams and lotions, and found that 41% of these

Table 4 Results of the physicochemical tests and reference values.

Results and reference values for each formulation					
Criteria	Biocilin gold hair tonic	Biocilin antidandruff hair tonic	Biocilin antidandruff shampoo	Biocilin gold antidandruff shampoo	Biocilin antidandruff conditioner
pH	4.88 (4.0–6.0)	5.47 (4.0–6.0)	6.02 (5.5–7.5)	5.56 (5.0–7.0)	4.14 (3.0–5.5)
Color	Adequate (yellow)	Adequate (dark blue)	Adequate (blue)	Adequate (light blue)	Adequate (blue)
Odor	Adequate	Adequate	Adequate	Adequate	Adequate
Aspect	Adequate (Clear liquid)	Adequate (Clear liquid)	Pearly and viscous	Opaque and viscous	Medium viscosity emulsion
Alcohol content	47 °GL (45–51)	49 °GL (45–51)	NA	NA	NA
Viscosity	NA	NA	17,310 (10,000–18,000) RPM	13,705 (10,000–30,000) RPM	27,420 (10,000–30,000) RPM

NA: not applicable; RPM: rotations per minute. Data in parenthesis represent the reference value from the manufacturer.

products were contaminated in exceeding levels according to the legislation. Factors such as the microbiological quality of water, raw materials and even the hygienic and sanitary conditions of the industries may influence these results.

3.3. Planktonic fungal cells susceptibility to the formulations

The tested formulations inhibited fungal growth; however, the efficacy was variable. Most of the studies with cosmetic formulations have been performed with voluntary people, and although these studies are very important for sensorial characteristics of the products, results on effectiveness might be influenced by the perception and satisfaction on using the product, which fluctuate over time and with the personal experience of treatment (Gupta and Nicol, 2004; Sridevi, 2014). To overcome this, we employed *in vitro* methods for antifungal activity, and explored biofilm formation by the first time.

The mean diameters of inhibition zones seen for the tested formulations are presented in Table 5. We used *M. furfur*, *T. rubrum* and *T. mentagrophytes* strains for these tests, which are known pathogens regarding dandruff and ringworm respectively. One may ask why we also employed a *C. albicans* strain for these tests, given that scalp infections by this yeast are rare. Antidandruff formulations can not only relieve the flaking and itching symptoms of dandruff, but extend their effects to the skin as well. This kind of cosmetic formulation is often prescribed in Brazil and other countries for skin infec-

tions by *Candida* as an accessible and low-cost alternative to complement the conventional treatment (Chimelli et al., 2003; Purim et al., 2009). Therefore, it was of interest to investigate if the formulations could be effective against this yeast.

Interestingly, despite the hair tonics sharing the same active ingredient in the same concentration, results were statistically different. The Gold Hair Tonic was significantly more efficient than the Hair Tonic against *T. mentagrophytes* planktonic cells ($p < 0.05$); nevertheless, the results of the Hair Tonic indicated that the efficiency of this formulation was higher than the Gold Hair Tonic against *C. albicans*, *T. rubrum* and *M. furfur* ($p < 0.001$). The differences in the composition of the formulations as a whole may help to explain this result: the Hair Tonic is also composed of phytoextracts of *Aloe vera* and *Arctium majus*, which have known antimicrobial and antifungal activities; the Golden Hair Tonic, on the other hand, contains hydrolyzed lupine protein, whose function is related to hair hydration and not to antifungal activity. But as the concentrations of these components are not available at the label, we can not speculate about their possible contributions to this result.

When analyzing the shampoos, it was seen that the Gold Antidandruff Shampoo formulation was more efficient against *C. albicans* and *M. furfur* than the Antidandruff shampoo ($p < 0.001$). On the other hand, the Antidandruff Shampoo was more efficient against *T. rubrum* than the Gold Antidandruff Shampoo ($p < 0.05$), and differences in inhibition zones

Table 5 Mean diameters of inhibition zones of each cosmetic formulation.

Cosmetic formulations		Mean diameters of inhibition zones			
		<i>C. albicans</i>	<i>T. rubrum</i>	<i>T. mentagrophytes</i>	<i>M. furfur</i>
Test 1	Biocilin hair tonic	21.5 ± 1.23**	22.75 ± 1.25**	10.75 ± 1.22	22 ± 0.81**
	Biocilin gold hair tonic	15.25 ± 0.95	5.75 ± 1.03	18.75 ± 2.90*	20.25 ± 0.95
Test 2	Biocilin antidandruff shampoo	13.75 ± 1.21	34 ± 1.07*	26.25 ± 0.95	21 ± 1.41
	Biocilin gold antidandruff shampoo	27 ± 1.4**	27.75 ± 1.02	31.5 ± 1.04	31 ± 1.41**
Test 3	Biocilin antidandruff conditioner	4 ± 2.69	6.75 ± 2.56	21.75 ± 0.95	–

Cosmetic formulations were compared in pairs, except the conditioner, which has only one formulation. Legend: (–): No inhibition zone observed. ANOVA and Tukey tests were used for this analysis.

* Significant difference ($p < 0.05$).

** Highly significant difference ($p < 0.001$).

formed in *T. mentagrophytes* experiments were not statistically significant. Apparently, the combined antifungal effect of salicylic acid and zinc pyrithione is the explanation for the better result seen when compared to piroctone olamine tested alone (Saple et al., 2000).

Unexpectedly, the inhibition zones from the conditioner were noticeably lower than all other formulations, and no inhibition zone was seen when testing against *M. furfur*. A possible explanation for that can be the low concentration of the active ingredient, reduced to half in the preparation of dispersions in sterile PBS containing polysorbate 80.

Variances in the total composition and the 10-fold difference of concentration of the active ingredients of the formulations we tested are items that help to explain this result. In contrast to this study, a study carried out by Schmidt-Rose et al. (2011) compared the *in vitro* efficacy of a shampoo containing 0.5% piroctone olamine and 0.45% climbazole with a shampoo containing 1% zinc pyrithione against *M. furfur* CBS 1878. The inhibition zone assay experiments performed indicated that both shampoos exhibited comparable antifungal activity in contrast to the free vehicle.

3.4. Golden shampoo and hair tonic effectively eradicated biofilms of dermatophytes

Considering the potential antifungal activity of the formulations against planktonic cells, we hypothesized that fungal biofilms could be eradicated *in vitro* in a widely accepted experimental model using 96 wells non-treated polystyrene plates. To test this hypothesis, we used a modified method from Dias-Souza et al. (2013), and observed that the golden shampoo and the hair tonic were effective against the biofilms from the strains tested, and the other formulations could not disrupt the formed biofilms of any strain (data not shown). These formulations were the most effective against the planktonic cells of the dermatophytes. The ineffectiveness of the other formulations in this test is not fully explained by their composition, although the susceptibility of the reference strains would be involved somehow.

Biofilms are complex surface-associated cell populations embedded in an extracellular matrix (ECM) of different phenotypes compared to planktonic cells like drug resistance, what happens due to biofilm structural complexity and metabolic heterogeneity (Kagan et al., 2014). Microorganisms hardly live as free (planktonic) cells, especially in infections, when biofilms are generally polymicrobial, although in some cases, it can be dominated by one or two microbial species. Developmental phases of biofilms are orchestrated by complex molecular events that culminate in microcolonies formation. The poor efficacy of antifungal agents remains a challenge in managing patients with fungal biofilms, independently of the etiological agent (Dessinioti and Katsambas, 2013; Costa-Orlandi et al., 2014). To our knowledge, the potential of biofilm eradication by cosmetic formulations is described for the first time.

In choosing suitable methods, preference was given to those that would give the most striking results. Nevertheless, this methodological approach is not free from limitations. Despite the fact that the biofilm formation and eradication models we used are widely accepted, fungal biofilms require several days to reach confluent growth in polystyrene plates (except

C. albicans). Also, a 50% solution was used for the reasons mentioned in the previous section. Thus, it remains unclear how these formulations would work in earlier stages of biofilm formation by these fungi.

4. Conclusion

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. Our data open doors in cosmetics microbiology by showing antifungal activity of cosmetic products against dermatophytes biofilms in a simple *in vitro* model. This study also opens doors for new *in vitro* assays with clinical isolates of dermatophytes, and also suggests the implementation of biofilm assays for pharmaceutical development of antidandruff formulations.

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