Received: 30 October 2023 | Revised: 11 December 2023 | Accepted: 16 January 2024

DOI: 10.1111/jocd.16219

JCD Journal of Cosmetic Dermatology WILEY

ORIGINAL ARTICLE

Evaluation of novel cosmetic shampoo formulations against *Malassezia* species: Preliminary results of anti-dandruff shampoo formulations

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Abstract

Objectives: *Malassezia* species are common, clinically relevant, and lipid-dependent yeasts of humans. They are also the leading causes of the dandruff problem of humans, and the azoles are used primarily in their topical and systemic treatment. Resistance to azoles is an emerging problem among *Malassezia* sp., which indicates the need of new drug assessments that will be effective against dandruff and limit the use of azoles and other agents in treatment. Among them, the efficacy of various combinations of piroctone olamine and climbazole against *Malassezia* sp. is highly important. Here, we assessed the efficacies of various piroctone olamine and climbazole formulations against *Malassezia* sp. in comparison with ketoconazole.

Methods: A total of nine formulations were included in the study, where each formulation was prepared from different concentrations of piroctone olamine and climbazole and both. All formulations contained the same ingredients as water, surfactants, hair conditioning agents, and preservatives. *Malassezia* furfur CBS1878, *Malassezia globosa* CBS7874, and *Malassezia sympodialis* CBS9570 were tested for antifungal susceptibility of each formulation by agar diffusion method. Sizes of the inhibition zones were compared with standard medical shampoo containing 2% ketoconazole, and the data were analyzed by Dunnett's multiple-comparison test.

Results: For all *Malassezia* sp. strains, climbazole 0.5% and piroctone olamine/climbazole (0.1%/0.1% and 0.1%/0.5%) combinations were found to have the same effect as the medical shampoo containing 2% ketoconazole. Piroctone olamine/climbazole 1.0%/0.1% formulation showed the same efficacy as 2% ketoconazole on *M. furfur* and *M. sympodialis*, while 0.1%/0.5% formulation to only *M. furfur*. For *M. globosa*, none of the formulations tested were as effective as ketoconazole.

Conclusion: The species distribution of *Malassezia* sp. varies depending on the anatomical location on the host. According to the results of this study, climbazole and piroctone olamine combinations seem to be promising options against the dandruff problem with their high antifungal/anti dandruff efficacy.

KEYWORDS
climbazole, dandruff, hair treatment, *Malassezia* sp., piroctone olamine

1 | INTRODUCTION

Inflammatory or non-inflammatory clinical manifestations due to *Malassezia* species, which are lipid-dependent yeasts, have been emerging. There are more than 18 described *Malassezia sp.* today, which are mostly present as commensal members of the skin microbiota, according to the age of the individual. *M. globosa* and *M. restricta* are the predominant species on the scalp, while *M. furfur* and *M. sympodialis* have been isolated more and more as causative agents of fungemia.[1–4] Regarding the sampling method and age-based variations (starting from the newborns), isolation rates of *Malassezia* sp. are reported within 5%–100%, according to duration.[5–8] *Malassezia* sp. is common in the etiology of head and neck dermatitis, seen after puberty as a subtype of atopic dermatitis. Type I hypersensitivity reaction is the expected pathogenic mechanism against *Malassezia* sp. Similarly, involvement of *Malassezia* sp. is common in the etiologies of both seborrheic dermatitis and *Pityriasis versicolor* infestation. Dandruff and seborrheic dermatitis are common diseases of the scalp that have basically the same histological structure.[9]

Geographical and ethnic differences in the distribution of *Malassezia* in healthy individuals and as disease agents are reported.[10] The incidence of seborrheic dermatitis/dandruff ranges from 30% to 95%, which is due to the lifestyle, habits, climate, and genetic factors, and the causative agents vary according to the location.[2,7,10] Studies conducted in Türkiye have revealed that *M. globosa*, *M. obtusa*, and *M. furfur* were the most reported *Malassezia* sp. of the skin.[11–13] In dermatological lesions such as pityriasis versicolor, *M. sympodialis* is considered predominant in the northern and southern United States, while it is also a leading species in the northern hemisphere.[10,14] However, it has been observed that dandruff was correlated more with *M. restricta*, while a lower *M. restricta* to *M. globosa* ratio was associated with a healthier scalp. In general, in the etiology of dandruff, a high association with many different *Malassezia* species within the mycobiome has also been observed.[15] Although *M. restricta* is at the forefront in the etiology of dandruff, it is observed that it is not primarily isolated in Türkiye. *Malassezia* sp. is known as the predominant fungi on the mycoflora of the scalp.[4] Elevation of unsaturated fatty acid levels is a predisposing factor associated with *Malassezia* sp. reproduction capacity, while the metabolites of *Malassezia* sp. may significantly promote dandruff formation.[4,16,17] Extreme colonization of *Malassezia* sp. due to the predisposing factors may also increase the dandruff formation, which indicates the need for effective, various treatment protocols and formulations in clinical management.[18–20] New therapeutics have been presented lately for the treatment of *Malassezia*-associated skin lesions and dandruff. Effective skin penetration and contact duration are common mechanisms in anti-dandruff shampoos.[18,20] Treatment strategies that aim to control hypersensitivity reactions on the skin target primarily the lowering the yeast colonization on the scalp. Such treatment protocols include single or combined applications of antifungals and anti-inflammatory agents. Alleviation of fungal dysbiosis is also important, especially when the ratios between *Malassezia* species are varied. Wang et al.[21] reported that glycyrrhetinic acid shampoo may be noteworthy as both a therapeutic and maintenance regimen for scalp seborrheic dermatitis as it reduces the number of both *M. restricta* and *M. globosa* in addition to classical antifungal treatment options. Lately, there has been an increasing number of research studies that assess the efficacies of various natural and synthetic products to develop alternative treatment agents for these patients.[3,21–23] Anti-dandruff shampoos may be either medical or cosmetic; medical shampoos contain antifungals alone, such as ketoconazole (KTZ), while the cosmetic anti-dandruff shampoos may contain three approved anti-fungal active compounds, which are selenium sulfide, piroctone olamine (PRT), or climbazole (CLZ).[18,24–30] Zinc pyrithione was also another ingredient of cosmetic shampoos until it was banned by the European Union in 2022. Considering the ban risk also for the other three anti-fungal active compounds, studies are required to confirm not only their efficacy but also their non-hazardous nature to human health.

It was well documented that application of shampoos that contain zinc pyrithione, KTZ, PRT, and CLZ to scalp lowers both *Malassezia* sp. content and skin flaking, which is a common disorder.[4,31] PRT is a common ingredient of various dermatological anti-dandruff formulations.[20,31] It has been reported that different topical formulations exhibit different levels of skin penetration for PRT. Its physicochemical structure is still under investigation, but PRT's skin penetration is known to be modified with different excipient and solvents.[32] Another ingredient, CLZ, has been present in different formulations of anti-dandruff treatment. Showing good penetration to the upper layers of epidermis, CLZ was found to be effective in formulations with or without piroctone.[31,33–35] Unveiling the safety levels and efficacies of both PRT and CLZ is highly essential, after zinc pyrithione was banned and the treatment options became limited. The aim of the present study was to assess the efficacies of different concentrations of PRT and CLZ on various standard *Malassezia* species in vitro.

2 | MATERIALS AND METHODS

2.1 | Formulations and study design

A total of nine formulations were assessed in the study. One of them was a shampoo base without any active agents. Two active agents (piroctone olamine [PRT] and/or climbazole [CLZ]) were combined to form a total of seven formulations in the base shampoo. In addition, a

medical shampoo was included in the study. The base shampoo consisted of: water, *sodium laureth sulfate*, *cocamidopropyl betain*, *glycerin*, *sodium chloride*, *decyl glucoside*, *parfum*, *benzyl alcohol*, *carbomer*, *glycol distearate*, *menthol*, *dimethiconol*, *TEA-dodecylbenzenesulfonate*, *guar hydroxypropyltrimonium chloride*, *cocamide MEA*, *methylchloroisothiazolinone*, *methylisothiazolinone*. All materials were added to water and dissolved at room temperature. A carbomer resin was used for thickening. The process is completed by adjusting the pH between 5.2 and 6.0. The medical anti-dandruff shampoo was purchased and used for the study. The details of the formulated shampoos are listed in Table 1.

2.2 | Mycological procedures

Three different standard *Malassezia* strains isolated from the dandruff (*Malassezia furfur* CBS1878, *Malassezia globosa* CBS7874, and *Malassezia sympodialis* CBS9570) were included in the study. All strains were subcultured in mDixon agar medium within 4 days at 33°C. The yeasts were added to sterile saline solution according to 0.5 McFarland level, and mDixon agar medium was spread over these yeasts using a sterile swab. To avoid humidity in the medium, all samples were kept at room temperature for 15 min. Discs having 6 mm of diameter were prepared using Whatman No. 1 filter paper and sterilized at 140°C for an hour. These sterile disks were immersed in the samples for 5 min in non-septic conditions. KTZ (15 μg/disc) was assessed as the positive control in the test with the same method (Table 1).[36] Discs were then immersed in Petri dishes with yeast using sterile swabs. All plates were monitorized for 4 days in humid environment at 33°C, and their inhibition zones were checked in mm visually in the end of incubation. All tests were triplicated, and their results were recorded daily.

2.3 | Statistical analysis

Statistical analysis was performed with the Minitab® (Ver 16.1.1, Cologne, Germany). One-way analysis of variance (ANOVA) with Dunnett's multiple-comparison tests were implemented to compare the inhibition zone of different *Malassezia* strains with both the base experimental formula (PO00CL00) and 2% medical shampoo containing KTZ (MS2KTZ). p values of <0.05 were considered as significant.

3 | RESULTS

On Day 4, relatively prominent inhibition zones were detected around the discs. The inner zone area was considered as the exact limit of inhibition. Sharp cut-off values were observed by naked-eye evaluation (Figure 1). The inhibition zone of each experimental formula on three *Malassezia* species were shown in Figure 2. According to the base formulation without PRT and CLZ as active substances; PO01CL00 and PO10CL00 were not different from the base, while the other formulations analyzed were more effective than the base. For all *Malassezia* sp. strains, PO00CL05, PO01CL01, PO01CL05, and PO10CL01 were found to have the same efficacy as MS2KTZ ($p > 0.05$), while other formulations showed lower activity (Figure 2). The formulation PO10CL01 showed the same effect for *M. furfur* and *M. sympodialis* as the medical shampoo containing 2% KTZ, while PO01CL05 showed the same effect for *M. furfur* ($p > 0.05$). For *M. globosa*, none of the formulations tested was as effective as MS2KTZ ($p < 0.05$).

4 | DISCUSSION

Today, hair shedding and hair thinning are two common problems associated with the dandruff.[37] Sunlight, together with commensal microorganisms, are the major environmental factors associated with these problems. It has long been known that seasonal variations and geographical differences may influence the lipid content of the skin.[17,38] With or without inflammation, the lipophilic *Malassezia* genus plays a key role in their pathogenesis, and reduction of these yeasts on the scalp is one of the main therapeutic targets.[3,4,31,37] KTZ, CLZ, and PRT are common agents that are active against

TABLE 1 Studied formulations and their properties.

No	Code	Shampoo	Piroctone Olamine % (w/w)	Climbazole % (w/w)	pH/Viscosity (cP)
1	PO00CL00	Base Shampoo	–	–	5.4/7920
2	PO01CL00	Experimental Formula	0.1	–	5.4/8320
3	PO10CL00	Experimental Formula	1	–	5.6/7560
4	PO00CL01	Experimental Formula	–	0.1	5.3/10580
5	PO00CL05	Experimental Formula	–	0.5	5.6/9800
6	PO01CL01	Experimental Formula	0.1	0.1	5.6/10200
7	PO01CL05	Experimental Formula	0.1	0.5	5.7/8840
8	PO10CL01	Experimental Formula	1	0.1	5.7/8100
9	MS2KTZ	Medical shampoo (Ketoconazole 2%)	–	–	–

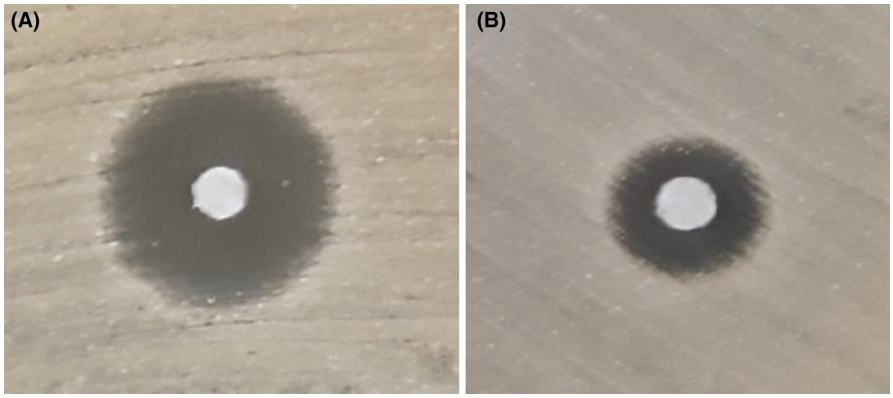

FIGURE 1 Inhibition zones of disc diffusion tests for *Malassezia furfur* CBS1878 and *Malassezia sympodialis* CBS9570 samples on mDixon agar (disk diameter: 6 mm).

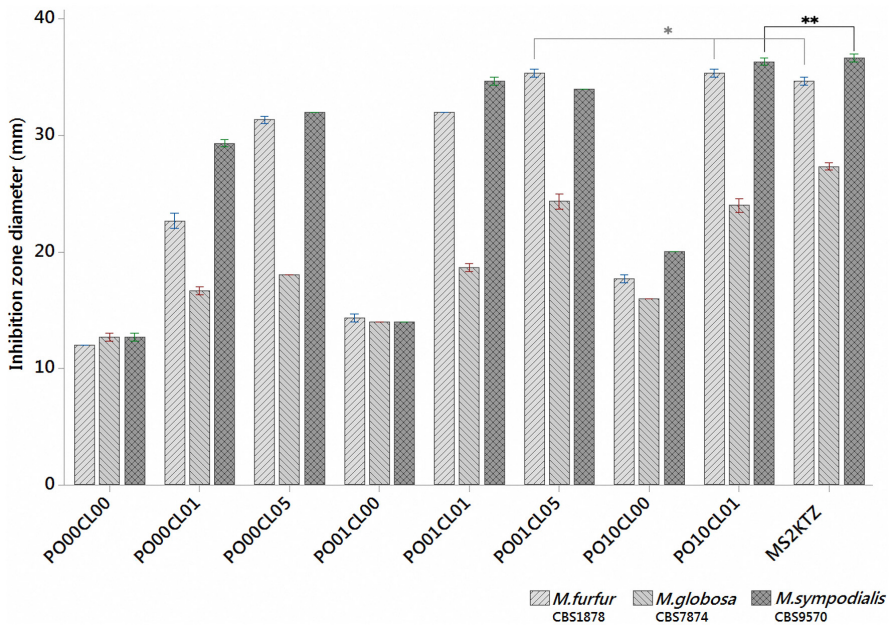

FIGURE 2 Inhibition zone diameter of the tested samples of the strains in the study. PO10CL01 for (**) *Malassezia sympodialis*, and both PO01CL05 and PO10CL01 are as effective as medical 2% ketoconazole (MS2KTZ) formulation on (*) *Malassezia furfur* (*, ** = $p > 0.05$).

Malassezia sp.; however, the outcomes of their application are mostly based on clinical interpretations since standard antifungal testing is not possible due to the lipophilic nature of *Malassezia* sp.[4,31,33,34,37] It is suggested that testing of not only the physicochemical characteristics but also the antifungal efficacies of therapeutics is crucial. Broth microdilution is mostly preferred for antifungal testing; however, due to the testing of surfactant-like products in dermatological treatment assessments, which may be confusing with microdilution, diffusion-based tests should be chosen.[39] It is shown that the standard dilution assays are suitable for the non-lipophylic *M. pachydermatis*; however, disk diffusion tests may be useful for the lipophilic *Malassezia* species according to the characteristics of the tested agent in lipophilic conditions, while they are not suitable for all antifungals.[36,40] Assessments with KTZ revealed no linear correlation between the disk diffusion and liquid microdilution. In the present study, KTZ levels in each three standard *Malassezia* sp. was found to be as expected regarding the study of Rojas et al.[36] The disk diffusion method used in the present study as described by Pooja et al.[40] supports the validity of the method of our study.

It was observed that PRT, CLZ, and their combinations with different ratios in the shampoo formulas that were added in different concentrations managed to form inhibition zones under the same conditions. It is possible to claim that the diffusion capacities of tested chemicals may be insufficient to determine true results. Since there are no disk diffusion test results with PRT and CLZ in the limited literature, it is not possible to compare our results at the moment. Yet, remarkable inhibition zones were determined in all tested *Malassezia species* against both PRT and CLZ, which may be regarded as sensitive, in our study. Regarding *M. furfur* and *M. sympodialis*, different cosmetic shampoo formulations of both PCT and CLZ were found to be as effective as KTZ in the present study, while this was not shown against *M. globosa* (Figure 2). Although *M. globosa* is generally accepted as a causal agent of dandruff, the species distribution of *Malassezia* sp. varies depending on the geographical location of the host.[2,10,14,41] Therefore, formulations showing activity against *M. furfur* and *M. sympodialis* have the potential to be effective in therapeutic approaches against dandruff in a wide geographical area. Results of the present study clearly indicate that antifungal activity is achieved with increasing concentrations of both PRT and CLZ added to the base shampoo. Highest inhibition rates were observed with the combinations; the highest rate was in Sample 8 (PO10CL01; PRT 1% – CLZ 0.1%),

while the inhibition was no different than the one in Sample 6 (PO01CL01; PRT 0.1% – CLZ 0.1%) combination. These results are comparable with Sample 9 (MS2KTZ; KTZ 2% w/w Shampoo), which contains only KTZ. Considering the *Malassezia* sp. studied, PRT alone (PO01CL00, PO10CL00) had an inhibitory effect, but less than the effect of CLZ alone (PO00CL01, PO00CL05). The combination forms had a remarkable inhibitory effect on all three *Malassezia* sp.

Previously, it has been shown that 2% climbazole shampoo lowered the number of *Malassezia* sp. on the dogs.[42] Clinical efficacy was reported with combination formulas, as well.[31,34] KTZ is an old antifungal agent of the azole group; it has long been sold in the market worldwide as a commercial medical shampoo. There is a common resistance to the azole group antifungals in systemic *Malassezia* infections. However, this is not yet common among the *Malassezia* sp. isolated from the skin,[43–46] although gene mutations have recently been demonstrated in *M. globosa* and *M. sympodialis*, which were associated with lower sensitivity to azole-group antifungals.[46] Development of new cosmetic shampoos that contain PRZ-CLZ combination is expected to lower the use of azole group antifungals. This will also prevent the spread of azole resistance in the community as well. The decision of European Union Regulatory Board about the prohibition of zinc pyrithion in anti-dandruff shampoo formulations is highly important as it creates an urgent need worldwide to develop new commercial products with available, effective, and non-restricted active ingredients against *Malassezia* sp. In the present study, CLZ and PRT, which are permitted by EU in the maximum concentrations of 0.5% and 1%, respectively, for rinse-off products (Regulation (Ec) No. 1223/2009 of the European Parliament and of the Council), were found to be effective against *Malassezia* sp. Further studies are warranted to confirm these findings.

5 | CONCLUSION

According to the results of this study, climbazole and piroctone olamine combination seem to be an effective option for a cosmetic shampoo with high antifungal/anti dandruff efficacy. New in vitro and in vivo studies are required to further support the efficacy of climbazole and piroctone olamine combinations.

DATA AVAILABILITY STATEMENT

The raw/processed data required to reproduce the above findings cannot be shared at this time as the data also forms part of an ongoing study.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is an in vitro study with known and approved fungal cultures. No animal or human experiment was involved.

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How to cite this article: Ergin Ç, Kurt Ö, Türkoğlu M, Sevinç H, Akbaba G. Evaluation of novel cosmetic shampoo formulations against *Malassezia* species: Preliminary results of anti-dandruff shampoo formulations. *J Cosmet Dermatol*. 2024;00:1-6. doi:10.1111/jocd.16219