

Antifungal and Anti-Biofilm Activities of Cosmetic Ingredients Against Clinical Dermatophyte Isolates

Cemre ÖZKANCA*, Sibel DÖŞLER**

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SUMMARY

The skin, with its low pH and sweat glands, acts as a physical barrier against microbial colonization. Dermatophytes, however, can develop resistance through biofilm formation and changes in membrane permeability. Cosmetic products applied to the skin may also contribute to this stress. This study evaluated the antifungal and antibiofilm effects of commonly used cosmetic raw materials against clinical dermatophyte isolates. Eleven isolates were tested: five *Microsporum canis*, four *Trichophyton rubrum*, and one each of *T. mentagrophytes* and *T. tonsurans*. Biofilm formation was assessed by crystal violet staining, antifungal activities by microbroth dilution, and antibiofilm activities against mature biofilms. Among the isolates, four *M. canis* were strong biofilm producers, one *M. canis* and two *T. rubrum* moderate, while others were weak. Minimum inhibitory concentrations ranged from 7.8–250 µL/mL for tea tree oil, 4–625 µL/mL for vitamin A, and 4–500 µL/mL for octocrylene, glycerin, or panthenol. Benzophenone-4 showed moderate activity (256–5000 µg/mL), whereas the UV filter DHHB was selectively active against *M. canis* (8–256 µg/mL). Vitamin A, glycerin, and octocrylene were most effective against *M. canis*, while panthenol and tea tree oil were more active against *T. rubrum*. Cosmetic ingredients reduced mature biofilms up to 3-log₁₀ cfu/mL, with tea tree oil, vitamin A, panthenol, Centella asiatica extract, and benzophenone-4 showing strong antibiofilm effects. These results indicate that cosmetic components possess significant antifungal and antibiofilm properties, offering a promising adjunct or alternative strategy for treating dermatophyte infections. Further research is needed to validate their clinical efficacy and safety.

Keywords: Cosmetic ingredient, dermatophyte, anti-biofilm, antifungal activity.

Kozmetik İçeriklerin Klinik Dermatofit İzolatlarına Karşı Antifungal ve Anti-Biyofilm Aktivitelerinin İncelenmesi

ÖZ

Deri, düşük pH değeri ve ter bezleri ile mikroorganizmaların kolonizasyonuna engel olmak üzere fiziksel bir bariyer sağlamaktadır. Dermatofitler, biyofilm oluşturma yetenekleri ve hücre zarı geçirgenliği değişiklikleri gibi çevresel stres faktörleri nedeniyle antifungal direnç geliştirebilmektedir. Cilde uygulanan kozmetik ürünler de bu strese katkıda bulunma potansiyelindedir. Bu çalışmada, yaygın kullanılan kozmetik hammaddelerin, klinik dermatofit suşlarına karşı antifungal ve anti-biyofilm etkinliğinin değerlendirilmesi amaçlanmıştır. Beş *Microsporum canis*, dört *Trichophyton rubrum*, birer *Trichophyton mentagrophytes* ve *Trichophyton tonsurans* olmak üzere toplam 11 suşun biyofilm oluşturma kapasiteleri kristal viyole boyama yöntemiyle belirlenmiştir. Antifungal aktiviteler mikrodilüsyon tekniği ile belirlenirken, etkili maddelerin antibiyofilm aktiviteleri olgun biyofilmlere karşı test edilmiştir. 11 suş arasında dört *M. canis* kuvvetli, bir *M. canis* ile iki *T. rubrum* orta ve diğer izolatlar zayıf biyofilm oluşturma özelliği göstermiştir. Çay ağacı yağı, A vitamini ve oktokrilen, gliserin ya da panthenol için minimum inhibitör konsantrasyonları sırasıyla 7,8-250, 4-625 ve 4-500 µL/mL arasında bulunmuştur. Benzofenon-4, 256-5000 µg/mL ile orta düzeyde aktifken UV filtresi DHHB, *M. canis*e karşı 8-256 µg/mL ile seçici olarak etkili bulunmuştur. Özellikle vitamin A, gliserin ve oktokrilen *M. canis*e karşı, panthenol veya çay ağacı yağı ise *T. rubrum*a karşı en aktif maddeler olarak bulunmuştur. Kozmetik hammaddeler, olgun biyofilmleri 3-log₁₀ cfu/mL'ye kadar inhibe etmiş olup, bu sonuçlara göre özellikle çay ağacı yağı, vitamin A, panthenol, Centella asiatica ekstresi ve benzofenon-4'ün güçlü antibiyofilm aktivitelerine sahip olduğu belirlenmiştir. Kozmetik bileşenler önemli antifungal ve antibiyofilm özellikler göstererek dermatofit enfeksiyonlarının tedavisinde umut verici bir alternatif yaklaşım sunmaktadır. Bu maddelerin klinik etkinlik ve güvenliğini doğrulamak için daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Kozmetik ürünler, dermatofit, anti-biyofilm, antifungal aktivite.

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* ORCID: 0000-0002-0342-2060, Hamidiye Vocational School of Health Services, The University of Health Sciences, Istanbul, Türkiye.

** ORCID: 0000-0001-5223-4755, Department of Pharmaceutical Microbiology, Istanbul University, Faculty of Pharmacy, Istanbul, Türkiye

INTRODUCTION

Dermatophytes are a group of organisms responsible for infections in keratin-rich tissues, including the skin, hair, and nails. Under normal circumstances, the skin does not provide a suitable environment for microorganisms to thrive due to its low pH and the presence of sweat glands (Blaak & Staib, 2018). However, under certain conditions, some infections can occur due to fungi in keratin-rich environments, colonizing the stratum corneum of the skin, hair shafts, and nails (Weitzman & Summerbell, 1995). These infections, which are commonly referred to as dermatophytosis, ringworm, or tinea, are prevalent worldwide and pose significant health concerns. Dermatophytes belong to three main genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*, and their infections manifest in various forms depending on the site of infection (Weitzman & Summerbell, 1995).

Since fungal cells are eukaryotic, like mammalian cells, generally a substance that is toxic to fungi can also be toxic to humans, and the development of new antifungal agents poses a serious problem (Nivoix et al., 2020). Besides the limited number of antifungal drugs, antifungal resistance is increasing worldwide and poses a serious problem for the treatment of clinically encountered infections. In clinical studies, a high rate of resistance is especially observed against the azole group, which is one of the main antifungal groups in the treatment of dermatophytes (Oliveira Carvalho et al., 2013). There are different mechanisms for fungal resistance, such as mixing the drug targets, increasing cell permeability, forming a biofilm, and stress triggering by the improper use of antifungal agents (Khurana et al., 2019; Revie et al., 2018). In the midst of the difficulties encountered in managing dermatophyte infections, biofilms -organized groups of fungal cells surrounded by a self-generated extracellular matrix that sticks to surfaces- are gaining more focus (Donlan, 2002). The ability of dermatophytes to form biofilms is crucial for their

survival and contributes to their resilience against antifungal therapies. *Trichophyton rubrum* and *Microsporum canis* are among the most frequently reported species to form biofilms, which protect fungal cells from antifungal agents and immune responses. These biofilms contribute to chronic infections and complicate treatment strategies (Costa-Orlandi et al., 2014; Markantonatou et al., 2023).

In dermatophyte infections, biofilms can develop on the skin, nails, and other areas, creating a protective environment for the fungi, which leads to several consequences such as heightened resistance, ongoing infections, and evasion of the immune system (Markantonatou et al., 2023). Consequently, the existence of biofilms calls for the formulation of innovative treatment approaches capable of effectively penetrating and disrupting these structures. Conventional antifungal therapies might be less effective against infections associated with biofilms. As a result, recent research is concentrating on discovering and developing new anti-fungal agents and/or combination therapies specifically targeting the biofilms.

While much of the research is dedicated to finding new treatments for resistant fungi, it's equally vital to examine how existing cosmetics influence biofilm formation. Cosmetics, which are applied to the skin's surface, are readily accessible as over-the-counter products. There are many types of cosmetics on the market that have moisturizing, protective, and caring effects (Halla et al., 2018), and create an environmental stress to fungi. Therefore, the addition of active cosmetic components like essential oils into topical products can present a viable approach for controlling dermatophyte infections. Their potential natural antifungal characteristics, along with their capacity to break down biofilms, render them valuable options for creating innovative therapies for dermatophytosis.

Recent studies highlight the importance of exploring alternative approaches, such as integrating antifungal agents into cosmetic formulations, to

address dermatophyte biofilms. These formulations may act as adjunct therapies, particularly for superficial infections, offering both antifungal activity and biofilm disruption (Halla et al., 2018). It has been established that several natural active compounds, especially essential oils, used in cosmetic formulations demonstrate encouraging antifungal effects against dermatophytes (Dall'oglio et al., 2015). The antifungal properties of these substances are linked to their capability to disrupt the fungal cell membrane and interfere with the production of vital cellular components. Additionally, UV filters such as benzophenone derivatives are recognized for their antimicrobial properties, possibly resulting from the generation of reactive oxygen species (ROS) when exposed to UV light. Some research indicates antifungal activity against organisms like *Candida albicans*, implying that these compounds may also hinder the growth of dermatophytes and the formation of biofilms (Hanson et al., 2006; Wang et al., 2013). This study aims to explore the antifungal and antibiofilm efficacy of certain raw cosmetic materials as a potential alternative or complementary strategy for treating dermatophyte infections.

MATERIALS AND METHODS

Microorganisms: A total of 11 clinical fungal isolates consisting of five *M. canis*, four *T. rubrum*, one *Trichophyton tonsurans*, and one *Trichophyton mentagrophytes*, obtained from specimens submitted to the Mycology Laboratories of Prof. Dr. Süleyman Yalçın City Hospital between January-March 2022. Identifications of all isolates were made by MALDI-TOF analysis. *T. rubrum* ATCC 28188 was used as a standard strain.

Tested substances: Natural products such as tea tree oil (TTO), *Hypericum perforatum* L. oil, *Centella asiatica* extract; UV filters in sunscreens such as benzophenone-4, octocrylene, ethylhexyl methoxycinnamate, diethylamino hydroxybenzoyl hexyl benzoate (DHHB); and glycerin, panthenol, collagen, hyaluronic acid, zinc oxide, vitamin A (VITA), and also standard antifungal itraconazole were

kindly provided by their respective manufacturers. Solid substances (e.g., benzophenone-4, *C. asiatica* extract, and VITA) were accurately weighed using a precision balance and dissolved in solvents suitable for their solubility properties following the manufacturer's recommendations. The solvents other than water were ethanol for *C. asiatica* extract, and DMSO for DHHB, ethylhexyl methoxycinnamate, *Hypericum perforatum* oil, octocrylene, TTO, and zinc oxide. All solutions were freshly prepared daily, and the concentrations were expressed as µg/mL for solid substances and µL/mL for liquids, consistent with standard practices in antifungal testing. To assess the potential antifungal effects of the solvents, solvent controls were also included in the experiments.

Media: Roswell Park Memorial Institute (RPMI)-tt1640 medium buffered with morpholine propane sulfonic acid (MOPS, Sigma-Aldrich), and adjusted to pH 7.0, was used for minimum inhibitory concentration (MIC) determinations and biofilm formations. Sabouraud Dextrose Agar (SDA) was used as a solid medium for mold growth and colony counts. Resazurin dye (0.1 mg/mL) was used for examining the growth of microorganisms.

Minimum inhibitory concentrations (MICs): For determining the antifungal activities of cosmetic substances, the Minimum Inhibitory Concentrations (MICs) against 11 fungal isolates were determined by the microbroth dilution technique according to the Clinical and Laboratory Standards Institute (CLSI) document M38, with minor modifications (CLSI, 2017). Two-fold serial antifungal dilutions in RPMI-1640 medium ranging from 5000 to 5 µg/mL for solid substances and 500 to 0.4 µL/mL for liquids were tested in a 96-well, U-shaped microplates. Each well was inoculated with fungi suspension diluted to give a final concentration of 5×10^3 cfu/mL, the trays were covered and placed in plastic bags to prevent evaporation and incubated at 30°C for 3-7 days. At the end of the incubation, 25 µL of resazurin dye was added to the wells, and after 1-3 h incubation, purple-colored wells were evaluated as growth

negative and pink colored wells as positive. The lowest concentration that inhibited the visible growth was determined as the MIC value. *T. rubrum* ATCC 28188 and itraconazole diluted from 64 to 0.06 µg/mL were used as a standard strain and standard antifungal, respectively.

Biofilm formation: A total of 11 fungal isolates were cultured on SDA medium, after incubation at 30°C for 7-14 days, 5 mL physiologically buffered saline (PBS) solution was added to the colonies, and molds were collected. The resulting suspension was added to 24-well flat-bottom cell-culture microplates to give a final concentration of 1×10^6 cfu/500 µL in RPMI-1640 medium and incubated at 30°C for 7 days. After the incubation, the medium was aspirated, and the wells were washed three times with 500 µL PBS to remove unattached fungi. To ensure the fixation of biofilms, 500 µL of 99% methanol was added to each well for 15 minutes, then methanol was aspirated, and plates were left to dry. 500 µL of 0.1% crystal violet dissolved in water was added to the wells, left for 5 minutes to stain, then the excess stain was aspirated, the wells were washed with tap water, and air dried. For re-solubility of the stain, 500 µL 95% ethanol was added, and the plates were incubated on an orbital shaker for 30 min. Then, the optical density was measured at 595 nm (BioTek EON ELISA reader). RPMI-1640 medium was used as a negative control, and *T. rubrum* ATCC 28188 standard strain was used as a positive control (Costa-Orlandi et al., 2014).

Determination of biofilm inhibition: The antibiofilm activities of cosmetic substances were tested against mature dermatophyte biofilms (Costa-Orlandi et al., 2014; Dos Santos & Dias-Souza, 2017) for the first time, the characteristics of *Trichophyton rubrum* and *T. mentagrophytes* biofilms. Biofilm formation was analyzed by light microscopy, scanning electron microscopy (SEM). According to the MIC results, 200 µL solutions of each active cosmetic ingredients were prepared in RPMI-1640 medium at a concentration of 10,000 µg/mL for solid substances and 1000 µL/mL for liquids and were added to the mature biofilms (described as above) in a 96-well, flat-

bottom tissue culture microtiter plates. After 7 days incubation at 30°C, the plates were washed twice with 200 µL PBS and placed in an ultrasonic water bath for 15 minutes to disrupt the biofilms. Then, the plates were stirred on a microplate vortex for 10 minutes, and 20 µL samples from each well, directly or after subsequent 1/10 dilutions, were plated on SDA. After the 3-7 days of incubation at 30°C, the colonies were counted. RPMI-1640 medium was used as a negative control, and *T. rubrum* ATCC 28188 standard strain was used as a positive control.

Statistical analysis: A bootstrap analysis was employed to estimate the mean $\log_{10}$ cfu/mL values and 95% confidence intervals (CI) for each cosmetic ingredient's antimicrobial effect against dermatophytes. This resampling strategy was chosen to address the impediments related to small test sizes, guaranteeing the vigor and unwavering quality of the factual results. A total of 1000 bootstrap emphases were performed for each group, and the confidence intervals were calculated at a 95% certainty level (Efron & Tibshirani, 1994). All experiments were performed duplicate, and in cases of any inconsistent results, a third replicate was conducted to confirm the findings.

RESULTS

Minimum inhibitory concentrations (MICs): According to the microbroth dilution test results, which were summarized in Table 1, the MIC ranges of cosmetic substances against fungal isolates were 4–250 µL/mL for TTO and glycerine, 4–62.5 µL/mL for VITA, followed by 4–500 µL/mL for octocrylene and panthenol. Benzophenone-4 showed moderate MIC activity with 256–5000 µg/mL, and the UV-filter DHHB was selectively active against *M. canis* with 8–256 µg/mL. Itraconazole was used as a standard antifungal for the standardization of the study, and its MIC values against *T. rubrum* ATCC 28188 standard strain were obtained within the quality control limits of CLSI throughout the study. Also, the solvent control results confirmed that the solvents at the highest concentrations, which used in the antifungal activity assays, exhibited no antifungal effect.

Table 1. Minimum inhibitor concentrations of cosmetic ingredients against dermatophytes ($\mu\text{L/mL}/\mu\text{g/mL}$)

	<i>M. canis</i> (n:5)	<i>T. rubrum</i> (n:4)	<i>T. mentagrophytes</i> (n:1)	<i>T. tonsurans</i> (n:1)
BEN	256-2500	1250-2500	625	5000
CEA	1250-5000	1250-2500	2500	5000
COL	-	-	-	-
DHNB	8-256	1250-2500	2500	5000
EMC	-	-	-	-
GLI	4-250	31-156	125	15.6
HPO	-	-	-	-
HYA	-	-	-	-
OCT	4-62.5	15-500	125	15.6
PAN	4-250	31-156	500	15.6
TTO	4-250	62.5-156	62.5	7.8
VITA	4-62.5	15-500	250	4
ZNC	-	-	-	-
ITR	0.125-1	0.125-0.5	0.03	1

BEN: Benzophenone-4, **CEA:** *Centella asiatica* extract, **COL:** Collagen, **DHNB:** Diethylamino hydroxybenzoyl hexyl benzoate, **EMC:** Ethylhexyl methoxycinnamate, **GLI:** Glycerine, **HPO:** *Hypericum perforatum* L. Oil, **HYA:** Hyaluronic acid, **OCT:** Octocrylene, **PAN:** Panthenol, **TTO:** Tea Tree Oil, **VITA:** Vitamin A, **ZNC:** Zinc oxide, “-”: No activity”

Biofilm formation: As shown in Table 2, among the total 11 dermatophyte isolates, four *M. canis* were strong biofilm formers, one *M. canis* and two *T. rubrum* isolates were moderate, and the others were weak biofilm formers.

Table 2. Biofilm-forming capacities of the isolates

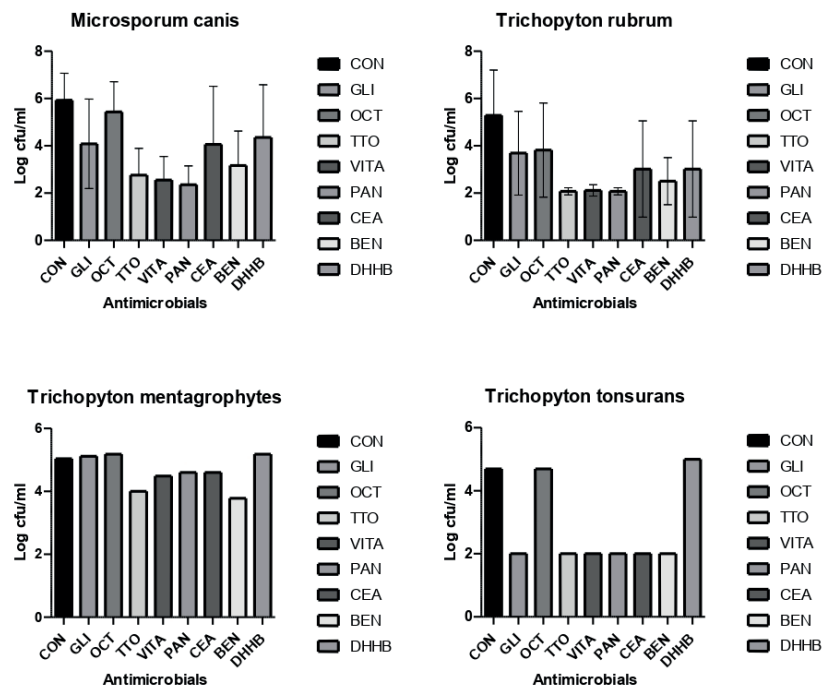
Dermatophyte isolates	Number of biofilm-forming isolates		
	Strong	Moderate	Weak
<i>Microsporum canis</i> (n:5)	4	1	-
<i>Trichophyton mentagrophytes</i> (n:1)	-	-	1
<i>Trichophyton rubrum</i> (n:4)	-	2	2
<i>Trichophyton tonsurans</i> (n:1)	-	-	1

Determination of antibiofilm activities: Against the mature biofilms of 11 dermatophyte isolates, the antibiofilm activities of cosmetic ingredients were summarized in Table 3. According to these results, as shown in Figure 1, cosmetic substances inhibited the mature biofilms up to 3-log_{10} cfu/mL. Especially TTO, VITA, panthenol, *C. asiatica* extract, and benzophenone-4 have strong antibiofilm activities.

Table 3. Antibiofilm activities of cosmetic substances

Cosmetic ingredients	Number of isolates showing the following log ₁₀ CFU/mL decrease at the 24 th hour											
	<i>M. canis</i> (n:5)			<i>T. mentagrophytes</i> (n:1)			<i>T. rubrum</i> (n:4)			<i>T. tonsurans</i> (n:1)		
	≥1	≥2	≥3	≥1	≥2	≥3	≥1	≥2	≥3	≥1	≥2	≥3
BEN	0	3	2	1	0	0	0	0	3	0	0	1
CEA	1	1	2	0	0	0	0	0	2	0	0	1
DHNB	1	1	1	0	0	0	0	0	2	0	0	0
GLI	1	1	2	0	0	0	2	0	1	0	0	1
OCT	2	1	0	0	0	0	1	0	1	0	0	0
PAN	0	1	4	0	0	0	0	0	3	0	0	1
TTO	0	2	3	1	0	0	0	0	3	0	0	1
VITA	0	2	3	0	0	0	0	0	3	0	0	1

BEN: Benzophenone-4, CEA: *Centella asiatica*, DHNB: Diethylamino hydroxybenzoyl hexyl benzoate, GLI: Glycerine, OCT: Octocrylene, PAN: Panthenol, TTO: Tea Tree Oil, VITA: Vitamin A.

**Figure 1.** Effects of cosmetic ingredients against the mature biofilms of dermatophytes.

CON: Control, GLI: Glycerine, OCT: Octocrylene, TTO: Tea Tree Oil, VITA: Vitamin A, PAN: Panthenol, CEA: *Centella asiatica*, BEN: Benzophenone-4, DHNB: diethylamino hydroxybenzoyl hexyl benzoate. The graph was created using the GraphPad Prism 7.0 program.

The results were expressed as the number of live fungi in biofilms as log₁₀ cfu/mL. Each bar is representative of the mean values of two independent tests, and error bars indicate the standard deviations

between isolates. Control bars indicate the viable cells in mature biofilms without any antimicrobial treatment.

Statistical analysis: Bootstrap analysis was chosen to address the limitations associated with small sample sizes and to enhance the robustness of the results; this method offers significant advantages over traditional parametric methods as it does not rely on parametric assumptions and provides reliable confidence intervals regardless of the data distribution characteristics. Among the tested compounds, TTO and VITA consistently demonstrated the lowest mean $\log_{10}$ cfu/mL values across all dermatophyte species, indicating strong antifungal and antibiofilm activities. For *M. canis*, TTO and VITA had mean $\log$ cfu/mL values of 2.0 (95% CI: 2.0–2.0). Similarly, TTO and VITA showed superior performance against *T. rubrum*, *T. mentagrophytes*, and *T. tonsurans*, with mean $\log$ cfu/mL values of 2.5 (95% CI: 2.5–2.5) and 3.0 (95% CI: 3.0–3.0), respectively.

In contrast, compounds such as benzophenone-4, DHHB, and *C. asiatica* extract exhibited higher mean $\log_{10}$ cfu/mL values, ranging from 5.5 to 7.0, suggesting limited antifungal efficacy. The narrow confidence intervals across all tested groups highlight the robustness of the results, despite the small sample size.

DISCUSSION

Dermatophyte infections are common fungal infections that are not life-threatening but decrease the quality of life because of their persistent behaviors, and they become particularly challenging to treat due to the formation of biofilms. These biofilms confer increased resistance to antifungal agents and contribute to the persistence and recurrence of infections. In clinics, generally oral or topical antifungals are used alone or in combination for the treatment of dermatophytosis, but studies show that the biofilm-forming makes it complicated, especially in nail infections (Fanning & Mitchell, 2012). For example, Danielli et al. demonstrated the biofilm-forming properties of *M. canis* isolates (Danielli et al., 2017). In another study, Costa-Orlandi et al. reported that *T. rubrum* isolates have more biofilm-forming

capacities than *T. mentagrophytes* (Costa-Orlandi et al., 2014). In this study, when we perform the biofilm formation assay for 11 clinical dermatophyte isolates, as shown in Table 2, especially *M. canis* isolates (4/5) were strong biofilm formers, while half of the *T. rubrum* isolates (2/4) formed moderate biofilms, and all others formed weak biofilms. These results are similar to other studies.

While research on antifungal agents that disrupt biofilms shows promise for treating infections, this study focused on investigating the antifungal and antibiofilm effects of cosmetic raw materials against clinical dermatophyte isolates. For this purpose, *H. perforatum* oil, TTO, *C. asiatica* extract, collagen, hyaluronic acid, VITA, and different UV filter' antifungal activities were examined against dermatophyte isolates. As shown in Table 1, while some raw materials have no antifungal activity, especially VITA, glycerin and octocrylene were active against *M. canis*, and panthenol and TTO against *T. rubrum* isolates, according to the MIC values. Also, TTO and benzophenone-4 were the most active agents against *T. mentagrophytes*, and VITA for *T. tonsurans*. It's thought that the differences might be a result of the fungal species differences and their clinical antifungal resistance properties.

Among the studied cosmetic ingredients, the substances that have antifungal activity against dermatophytes were used for the antibiofilm assays. Because of the biofilms' extracellular matrix provides protection for dermatophytes under undesirable conditions, antifungals and immune mechanisms (Pereira, 2021), the frequently used cosmetic raw materials might be an alternative treatment agent alone or in combination with antifungals. In this study, although the antibiofilm activities of these raw materials against dermatophyte biofilms vary depending on the substances and strain, especially TTO, VITA, panthenol, and benzophenone-4 can cause a $\geq 2 \log_{10}$ decrease from the survival cell counts, except for *T. mentagrophytes*. Besides them, while the glycerin and *C. asiatica* extracts were active against

biofilms of especially *T. tonsurans*, followed by the *T. rubrum* and *M. canis*, the DHHB had a limited effect against *T. rubrum* or *M. canis*, and octocrylene was generally not effective, except for *T. rubrum*.

TTO is one of the well-known natural antiseptic derived from *Melaleuca alternifolia* which is frequently used in various cosmetic products such as shampoos, massage oils, nail and hand creams, or detergents, and especially used for the treatment of vaginal candidiasis and dermatophytes (Hammer et al., 2002; Larson & Jacob, 2012). TTO contains several active components of monoterpenes and alcohols, such as terpinen-4-ol, which disrupt the fungal cell membrane, leading to increased permeability and leakage of cellular contents, which compromises the structural integrity of the fungal cell, ultimately causing cell death. Also, TTO components can cause oxidative stress in fungal cells by generating ROS, which can damage essential cellular components such as lipids, proteins, and DNA, and further compromise cell viability (Abd El-Salam et al., 2023; Fitriani & Surini, 2022). Additionally, the proliferation of fungal pathogens is effectively curtailed by TTO by disrupting the key enzymatic functions and cellular processes (Upadhyay et al., 2023). For example, Roana et al. showed that TTO has antimicrobial activity against *T. rubrum* isolates either alone or in combination with some antifungals (Roana et al., 2021). Similarly, we showed that TTO has antifungal activities against all studied dermatophytes, and it has strong antibiofilm activities on mature biofilms of *M. canis*, *T. rubrum*, and *T. tonsurans*.

VITAs are a group of unsaturated nutritional organic compounds, including retinol, retinal, and several provitamins, responsible for the biological activities (Beckenbach et al., 2015). Although VITA is included in many preparations that are used for the skin, especially for the treatment of acne as a face cream (Leyden et al., 2017). Besides its cosmetic effects, some studies showed that, it has an antifungal activity against a broad-spectrum of human opportunistic pathogens, for example Campione et al.

showed that retinoid, a product of VITA, is effective in the treatment of nail infections, especially caused by *T. rubrum* and *T. mentagrophytes* (Campione et al., 2015). But the mechanism underlying the inhibition of growth and proliferation of certain fungal species by retinoids remains unclear (Pistoia et al., 2022). In this study, VITA showed strong antifungal and antibiofilm activities against *M. canis*, *T. tonsurans*, and *T. rubrum*.

Panthenol is an active substance that is frequently used in dermatology for its moisturizing, emollient, and barrier properties, which protect the skin, and has some therapeutic properties (Pavlačková et al., 2019). Although panthenol has not been directly studied for its antifungal activity, its role in skin health can indirectly support antifungal treatments. For example, Chohnan et al. found that panthenol effectively inhibits the growth of staphylococci, which can complicate the fungal infections by damaging the skin barrier (Chohnan et al., 2014). Also, some studies have shown that panthenol increases the effectiveness of antiseptics such as chlorhexidine gluconate (Kramer et al., 2016). However, under normal conditions, panthenol is required for the growth of pathogenic microorganisms; in our study, it showed $>2 \log_{10}$ decrease in all dermatophyte biofilms, except for *T. mentagrophytes*. This might be due to the competition for interacting with another vitamin B pathway used by fungi (Saliba et al., 2005).

UV filters such as benzophenone-4, octocrylene, and DHHB are organic chemicals developed to protect the skin from the harmful UV rays of the sun by absorbing those rays and reducing the effects of direct sun exposure (Giokas et al., 2007). Because of their UV ray filtering properties, UV filters are widely used in sunscreens as a barrier that protects skin from the sun, and skin cancer (Silvia Díaz-Cruz et al., 2008). However, the antifungal activity of benzophenone-4 has not been extensively studied; similar benzophenone derivative bicyclic imides were found to have significant antimicrobial activities, including antifungal effects (Fadel & Al-Azzawi,

2022). Octocrylene is also an organic compound used in sunscreens and cosmetics. It is a viscous, oily liquid that is clear and colorless, and it can convert to benzophenone-4 through the retro-aldol condensation. Octocrylene can penetrate the skin as a photosensitizer, resulting in an increased production of free radicals under illumination (Hanson et al., 2006). While the antifungal properties of octocrylene is unclear due to limited direct evidence, in a study some poly (phenylene ethynylene)-based cationic conjugated polyelectrolytes (CPEs) and oligophenylene ethynylenes (OPEs) compounds, which have structural similarities with UV filters such as octocrylene, showed significant antifungal activity against *Saccharomyces cerevisiae* and *Candida albicans*, particularly when exposed to UV radiation (Wang et al., 2013).

To the best of our knowledge, there are no studies about all UV filter' antimicrobial or antifungal properties. In this study, it was found that UV filters benzophenone-4, octocrylene, and DHHB showed good antifungal properties against the studied dermatophytes, especially octocrylene, which has the lowest MIC values, except for *T. mentagrophytes*. According to antibiofilm assays, while benzophenone-4 decreases the survival of cells in biofilms by more than $\geq 2 \text{ Log}_{10}$, except for *T. mentagrophytes*, octocrylene and DHHB could only decrease the *M. canis* and *T. rubrum* biofilms by 1-2 Log_{10} .

Glycerin is a substance that is frequently used in many medical products as a therapeutic or in personal care/cosmetic products due to its fragrance, hair moisturizer feature, humectant, oral hygiene product, and skin protective properties (Becker et al., 2019). Especially its hygroscopic action causes it to extract water from the bacteria and fungi, hence preventing them from proliferation (Linser, 2002). It is also known that some substances obtained from glycerin have antibacterial and antifungal properties (Len et al., 1996). *C. asiatica* L., also known as *Gotu Kola*, is a plant used especially in South Asia,

showing antidiabetic, antioxidant, and wound healing properties (Chandrika & Prasad Kumarab, 2015), and its extracts are effective antimicrobial agents against fungi such as *Aspergillus niger* and *C. albicans* (Wong & Ramli, 2021). In this study, similar to other studies, glycerin and *C. asiatica* extract showed strong antifungal activity on a weight basis, against all studied dermatophytes, but they could only decrease the *T. tonsurans* biofilms by more than $\geq 2 \text{ Log}_{10}$. Against other biofilms, they have limited antibiofilm activities except for *T. mentagrophytes*.

The practical applicability of our findings may be impacted by the differences in the concentrations of cosmetic ingredients assessed in our antibiofilm assays compared with the usual levels in cosmetic formulations. For example, TTO and Panthenol were tested at 1000 $\mu\text{L/mL}$, in the 0.1% to 2% (1000 to 20,000 $\mu\text{g/mL}$) and 0.1% to 5% (1000 to 50,000 $\mu\text{g/mL}$) ranges, respectively, which are commonly used in skin care products for their antifungal properties (Carson et al., 2006; Ebner et al., 2002; Enshaieh et al., 2007). Considering the densities of TTO and Panthenol, it is thought that although it is more advantageous for TTO, these effective values remain within the usage limits for both substances. Similarly, glycerin is typically incorporated at 2% to 10% (20,000 to 100,000 $\mu\text{g/mL}$) as a humectant, and was assessed at 1000 $\mu\text{L/mL}$, within the safety limits (Fluhr et al., 2008). Unfortunately, VITA was also tested at 1000 $\mu\text{L/mL}$, exceeding the safer 0.01% to 0.1% (100 to 1000 $\mu\text{g/mL}$) range used in anti-aging and acne treatments (Mukherjee et al., 2006), which may limit its practical utility despite its potent antibiofilm activity. Benzophenone-4 tested at 10,000 $\mu\text{g/mL}$, align reasonably with their typical cosmetic ranges of 0.1% to 5% (1000 to 50,000 $\mu\text{g/mL}$) (Santos et al., 2012), supporting the relevance of their antibiofilm effects in formulations. Lastly, octocrylene and DHHB, tested at 10,000 $\mu\text{g/mL}$, are used in sunscreens at 1% to 10% (10,000 to 100,000 $\mu\text{g/mL}$) (Santos et al., 2012), hinting that their limited activity might improve at higher concentrations. These findings underscore

the importance of balancing efficacy and safety when adapting our results for cosmetic applications, with future studies needed to test concentrations more reflective of industry standards.

The results obtained through bootstrap analysis confirm the significant antifungal potential of TTO and VITA against dermatophyte biofilms. These findings align with previous studies reporting the potent antifungal properties of TTO and VITA. The low log cfu/mL values and narrow CIs suggest that these compounds could be promising candidates for developing innovative antifungal formulations. However, compounds like benzophenone-4 and *C. asiatica* extract demonstrated limited efficacy, emphasizing the need for further optimization or combination strategies to enhance their antifungal effects. Bootstrap analysis also validated the reliability of the findings, providing robust statistical support despite the inherent limitations of small sample size experiments.

The findings of this study suggested that incorporating cosmetic ingredients like TTO, VITA, and panthenol into topical formulations could provide a supplementary approach to managing dermatophyte infections. These products may serve as standalone options for mild infections or as adjunctive treatments alongside conventional antifungals to target biofilm-associated resistance. Clinical trials are essential to establish optimal concentrations, assess safety profiles, and confirm their efficacy in real-world applications (Campione et al., 2015; Halla et al., 2018). However, because the antibiofilm assays employed very high concentrations (1000 µL/mL or 10.000 µg/mL), direct translation of these levels into topical formulations could pose practical challenges—both in terms of skin tolerance (irritation, sensitization) and formulation stability (viscosity changes, emulsion breakdown).

As an overall result of the study, the antifungal and antibiofilm properties of TTO, VITA, panthenol, and benzophenone-4 were extremely high, followed by

the *C. asiatica* extract and glycerin against *M. canis*, *T. rubrum*, and *T. tonsurans* isolates. On the other hand, *T. mentagrophytes* was recorded as the most resistant isolate in this study. According to these results, we think that the substances used in cosmetic products could be effective in the treatment of persistent fungal infections and fungal biofilms on the skin. However, further research and clinical trials are essential to validate efficacy in clinical settings, evaluate the long-term safety to optimize their use, and establish standardized guidelines for their application in dermatology. Understanding the antifungal effects of cosmetic active ingredients can lead to innovative solutions for treating dermatophyte infections. Such studies can bridge the gap between cosmetic science and medical mycology, providing new avenues for both preventive and therapeutic applications.

This study is limited by its *in vitro* design, which may not fully replicate the complex environment of clinical infections. Also, one of the limitations of this study is the relatively small number of clinical isolates tested (n=11). These isolates were collected from specimens submitted to the Mycology Laboratories of Prof. Dr. Süleyman Yalçın City Hospital between January and March 2022. The primary reason for the limited number of isolates was the reduced patient admissions during this period, attributed to the ongoing COVID-19 pandemic, which significantly impacted healthcare service utilization (Moynihan et al., 2021). Consequently, the generalizability of the findings may be constrained, and further studies with larger and more diverse isolate sets are needed to validate the antifungal and anti-biofilm activities of the tested cosmetic ingredients. Further research is required to evaluate the long-term safety and efficacy of them through controlled *in vivo* and clinical trials.

AUTHOR CONTRIBUTION STATEMENT

SD and CÖ jointly developed the hypothesis for the study. CÖ conducted the experimental work. The manuscript was prepared collaboratively by both

authors, with shared responsibility for reviewing and finalizing the text.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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