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ORIGINAL ARTICLE / ÖZGÜN MAKALE

Novel microbiome friendly purifying oil cleanser formulation with oil-soluble postbiotics

Yağda çözünen postbiyotiklerle yeni mikrobiyom dostu temizleyici yağ formülasyonu

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Öz

Amaç: Son yıllarda hastalıklardan korunmada beslenmenin etkinliğinin yanında koruyucu kozmetik bakım da giderek önem kazanmıştır. Koruyucu yaklaşımda özellikle mikrobiyotanın etkinliği pek çok çalışmada yer almıştır. Mikrobiyotanın durumu, vücuttaki enflamasyon, çeşitli metabolik hastalıklar, dermal hastalıklar, nörodejeneratif hastalıklar veya dermal hastalıklarla ilişkilendirilmiştir. Bu çalışmada, yağ içinde çözündürülmüş postbiyotik hammadde kullanılarak yapılan temizleyicinin cilt mikrobiyotasına ve hücre üzerine olumlu etkilerinin araştırılması amaçlanmıştır. Bu çalışmada, %2 postbiyotik LTO 35 içeren cilt temizleyici yağın, normal keratinositler (HEKA 500K CELL) ve cilt mikrobiyota simülasyonu (Lactobacillus crispatus LTC-KC24011, Staphylococcus epidermidis ATA-LSE 0198052, Staphylococcus aureus ATA-LTCA 011204, Staphylococcus capitis ATA -LSC 0201201, Propionibacterium acnes ATA-LPC 0204221, Streptococcus pyogenes ATA TCSP 210911, Candida albicans ATA-LTCA 0504212) üzerindeki etkileri değerlendirilmiştir. Sonuçlar değerlendirildiğinde, keratinositlere zarar vermediği, mikrobiyota dengesini koruduğu ve hücresel onarıma destek olduğu görülmüştür. Bu çalışmada, yağ içinde çözündürülmüş postbiyotik hammadde kullanılarak yapılan temizleyicinin cilt mikrobiyotasına ve hücre üzerine olumlu etkilerinin araştırılması amaçlanmıştır.

Yöntem: Bu çalışmada, %2 postbiyotik LTO 35 içeren cilt temizleyici yağın, normal keratinositler (HEKA 500K CELL) ve cilt mikrobiyota simülasyonu (Lactobacillus crispatus LTC-KC24011, Staphylococcus epidermidis ATA-LSE 0198052, Staphylococcus aureus ATA-LTCA 011204, Staphylococcus capitis ATA -LSC 0201201, Propionibacterium acnes ATA-LPC 0204221, Streptococcus pyogenes ATA TCSP 210911, Candida albicans ATA-LTCA 0504212) kullanılmıştır. Simülasyonda kullanılan bakteriler ve formülasyon içinde kullanılan postbiyotik (LTO 35 Streptococcus thermophilus ATA-LTC St140700, Bifidobacterium animalis ATA-BSLA0310, Lactobacillus acidophilus ATA-LAP1201 ferment ekstrakt in oil lizat) ATA BIO Teknoloji kültür koleksiyonundan temin edilmiştir. Simülasyon kontak süresi 1 saat olarak uygulanmıştır.

Bulgular: Bu çalışmada kullanılan Purifying Oil Cleanser with Postbiotics numunesi, mikrobiyolojik açıdan uygun olarak değerlendirilmiştir. Denge testi olumlu bulunup, patojen yönünde değişmediği gözlenmiştir. Yapılan analizlere göre; Purifying Oil Cleanser with Postbiotics numunesinin cilt mikrobiyotası çeşitliliğine zarar vermediği ve mikrobiyom dostu olduğu ifade edilebilir.

Sonuç: Sonuçlar değerlendirildiğinde, keratinositlere zarar vermediği, mikrobiyota dengesini koruduğu ve hücresel onarıma destek olduğu görülmüştür.

Anahtar Kelimeler: Probiyotik, Postbiyotik, Mikrobiyota Dostu, Yağ Temizleyici, Kozmetik

INTRODUCTION

In recent years, research has increasingly highlighted the role of cutaneous microbiota in skin health and disease. Historically, cosmetic products were formulated for objectives such as cleansing, hydration, or protection against external factors; however, contemporary approaches that target the maintenance or enhancement of skin microbiota are gaining significance (1). In this context, the use of microbiota-focused ingredients such as prebiotics, probiotics, and postbiotics is of interest in the cosmetics sector.

Probiotics are defined as live microorganisms that provide benefits to the host, while prebiotics are selectively fermentable ingredients that support the growth or activity of the microbiota (2). Postbiotics are metabolic products of cellular components produced or secreted by probiotic microorganisms that do not require the presence of live microorganisms and provide benefits to the host (3). Postbiotics offer a more stable, standardisable, and safer alternative to the use of probiotics (3, 4).

Paraprobiotics (also termed inactive probiotics or “ghost” probiotics) and postbiotics represent alternative approaches to traditional probiotic use. Paraprobiotics are inactivated microbial cells or cell lysates that can provide host benefits when administered at adequate concentrations, while postbiotics are bioactive. Postbiotics are bioactive molecules (e.g., metabolites

and cellular fractions) synthesized by probiotic microorganisms that confer physiological benefits to the host. These approaches aim to overcome the limitations related to the viability, stability, and safety profile of probiotics and offer the potential for microbiome-targeted interventions (5,6).

The benefits of using postbiotics can be direct or indirect. For instance, postbiotics have been shown to maintain microecological and immune homeostasis by enhancing the interaction of the human organism with the microbiota (7, 8). Furthermore, positive effects on mental health can be achieved by improving the functioning of the brain-gut-microbiota axis. Postbiotics have also been demonstrated to exert direct benefits on host cells, while indirect benefits include the promotion of probiotics and the inhibition of pathogen growth. As with prebiotics, the effects of postbiotics vary depending on the type, strain and metabolic product of the microorganism (9). The most important effects of the postbiotic product SCFA are its anti-inflammatory and antioxidant properties. The present study sought to evaluate the effects of postbiotic LTO 35, obtained from *S. thermophilus, B. animalis and L. acidophilus* lysates used in an oil-based cleanser formulation, on human keratinocyte cells and simulated skin microbiota in *in vitro* models.

A study conducted by Aslan & Tarhan Celebi (2023) demonstrated that postbiotics specifically inhibit odour-causing microorganisms while concurrently supporting and balancing the natural axillary microbiota. The findings revealed that formulations containing *Lactobacillus* ferment lysate extract were effective in reducing unpleasant odours by normalising the microbiota (10). In a study conducted by Gökçe and Aslan on the pharmaceutical applications of postbiotics, the antimicrobial potential of liposomal postbiotics in gel formulations was investigated. The optimised gel (LG1) demonstrated effective antimicrobial activity that was comparable to that of free postbiotics against various pathogens, while providing advantages such as controlled release, stability and improved usability. These findings emphasise the potential of liposomal postbiotics for pharmaceutical applications (11). In a study by Tarhan Celebi et al., the treatment of colon cancer by postbiotic substances is examined. The viability of normal colon fibroblast cells damaged by TTX 100 is restored by buttermilk enriched with the postbiotic LTW 35, and the viability of colorectal cancer cells is reduced in a concentration-dependent manner, showing both reparative and anticancer effects. The observed decrease in Ca19-9 tumour marker levels further emphasises its potential to reduce tumour activity (12).

A plethora of academic studies have been conducted in the domain of nutricosmetics and cosmetics, encompassing a wide range of natural sources (13,14), essential oils (15) from hair care to skin care (16, 17). Moreover, even in the field of baby care, there exist studies that utilize natural sources and investigate their antimicrobial properties (18, 19). In addition to natural products, safety studies such as ADME (absorption, distribution, metabolism, excretion) of many semi-synthetic and fully synthetic active substances (20, 21) and studies on antibacterial activity have been conducted (22). However, these studies do not address

the subject of microbiome-friendly research on intestine, skin and skin microbiota.

The skin, the largest organ of the human body, is colonised by a variety of microorganisms. The majority of these microorganisms are harmless and even beneficial to their hosts. This colonisation is driven by the ecology of the skin surface, which is highly variable depending on environmental factors. In addition, the microbiota also functions in the education of the immune system (23). Keratinocytes in the skin represent a cellular compartment that is constantly renewed and the wound healing process is dominated by the interaction of keratinocytes with fibroblasts (24,25,26).

The practice of cleaning is an important daily activity that is associated with both skin diseases and general health. It is a relatively modern concept, coinciding with the mass use of commercial soap from the early 20th century (27). It is estimated that the bacteria in the human body far outnumber the human cells in an individual, and this community is termed the human microbiome. According to another view, various microbial communities that have fundamental roles in human health and disease are present in the human body (28).

The present study set out to evaluate the effects of postbiotic LTO 35, obtained from *S. thermophilus, B. animalis* and *L. acidophilus* lysates used in an oil-based cleanser formulation, on human keratinocyte cells and simulated skin microbiota in vitro models.

METHODS

In this research, a skin cleansing oil containing 2% postbiotic LTO 35 was utilised to simulate normal keratinocytes (HEKA 500K CELL) and skin microbiota (*Lactobacillus crispatus* LTC-KC24011, *Staphylococcus epidermidis* ATA-LSE 0198052, *Staphylococcus aureus* ATA-LTCA 011204, *Staphylococcus capitis* ATA -LSC 0201201, *Propionibacterium acnes* ATA-LPC 0204221, *Streptococcus pyogenes* ATA TCSP 210911, *Candida albicans ATA-LTCA 0504212*). The bacteria utilised in the simulation and the postbiotic employed in the formulation (LTO 35 *Streptococcus thermophilus* ATA-LTC St140700, *Bifidobacterium animalis* ATA-BSLA0310, *Lactobacillus acidophilus* ATA-LAP1201 ferment extract in oil lyzate) were obtained from the ATA BIO Technology culture collection in Türkiye. The duration of contact in the simulation was set at one hour.

MTT (In *vitro* cytotoxicity fest)

Tetrazolium salts, such as MTT, are frequently utilised in cell proliferation tests, which are based on the measurement of metabolic activity. The underlying principle is based on the measurement of the colour change in the absorption spectrum by an ELISA reader or a spectrophotometer, as a result of the increased dehydrogenase enzyme activity of proliferating cells. In this process, tetrazolium (MTT: yellow) is used to produce formazan (purple) dye. The cells will be cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin in an oven with 5% CO2 maintained at 37°C. When

cells reach 80% proliferation, they will be washed with phosphate-buffered saline (PBS) and trypsinized with 0.25% Trypsin-EDTA for passaging and seeding each time. Cells reaching sufficient proliferation will be used in the subsequent toxicity tests.

Table 1. Microorganisms representing Artificial Skin Microbiota and amount of utilisation.

Microorganism	İnoculum
Lactobacillus crispatus ATA-LTC 240522	3.50E+02
Staphylococcus epidermidis ATA-LSE 0198052	3.50E+02
Staphylococcus aureus ATA-LTCA 011204	3.50E+02
Staphylococcus capitis ATA -LSC 0201201	3.50E+02
Cutibacterium (Propionibacterium) acnes ATA-LPC 0204221	3.50E+02
Streptococcus pyogenes ATA TCSP 210911	3.50E+02
Candida albicans ATA-LTCA 0504212	3.50E+02

The environment has been modified to be conducive to the presence of artificial microbiomes.

Microbiological analyses

The formulation developed for microbiological analysis within the scope of the study was disinfected with 70% ethanol. To dissolve the product, 5 g/L polysorbate 80 was added to 90 ml TSP (Buffered Sodium Chloride Peptone), 10 g of sample was added and left to dissolve in a water bath for 10-15 minutes. Serial dilutions (10^{-2}, 10^{-3}, 10^{-4}) were prepared by transferring 1 mL of the sample suspension to 9 mL of TSP using the pour plate method. The dilutions were repeated twice by inoculating 1 mL of the diluted tubes into a 90 mm petri dish. Then 15-17 mL of agar medium cooled to 45°C in a water bath was poured into the petri dishes and left to freeze. Tryptic Soy Agar (TSA) was used for the total number of aerobic mesophilic microorganisms which were left at 30-35°C for 3-5 days. SDA medium was used for total yeast and mould counts and the media were incubated at 20-25°C for 5-7 days. In case of growth, the calculation formula is used to count colonies visible to the naked eye. This formula is as follows:

CFU/ml = Total number of colonies obtained x dilution factor/ Sample volume

Enrichment

10 g of the sample dissolved in buffered sodium chloride peptone was transferred to 90 mL of Tryptic Soy Broth (TSB). This medium contains lecithin and polysorbate required for neutralisation and is a general producer medium. After thorough shaking, the medium was incubated at 30-35°C for 18-24 hours (maximum 72 hours). After incubation, a selective medium was used. Enrichment was performed for *E. coli, P. aeruginosa and S. aureus*. For *C. albicans*, 10 mL (1 g or mL) of the sample dissolved in TSP was transferred to 90 mL Sabouraud Dextrose Broth (SDB). After shaking well, it was incubated at 30-35°C for 72 hours (maximum five days). After incubation, a selective medium was used.

Investigation of Aerobic Mesophilic Bacteria

After enrichment, 1 mL of TSB medium was taken and placed in sterile petri dishes. 5 mL of medium was added to Tryptone Glucose Extract Agar (TGEA) medium cooled to 45°C, mixed and inoculated with the sample in duplicate and allowed to solidify. After solidification, it was incubated at 37°C for 48 hours. In case of growth at the end of incubation, the number of colonies formed is calculated taking into account the dilution factor.

Investigation of The Presence of *Escherichia Coli*

After enrichment, 1 mL of TSB medium was taken and placed in sterile petri dishes. Then 5 ml of medium was added to Macconkey Agar (MCA) medium, mixed and the medium was inoculated with the sample in duplicate and allowed to solidify. Incubated at 30-35°C for 24 hours (maximum 48 hours).

Investigation for The Presence of *Staphylococcus Aureus*

After enrichment, 1 mL of TSB medium was taken and placed in sterile petri dishes. 5 ml of medium was added to Mannitol Salt Agar (MSA) medium cooled to 45°C, inoculated with the sample in duplicate and allowed to solidify. Incubated at 30-35°C for 24 hours (maximum 48 hours).

Investigation of The Presence of *Pseudomonas Aeruginosa*

After enrichment, 1 mL of TSB medium was taken and placed in sterile petri dishes. 5 mL of medium was added to Cetrimide Agar (CA) medium cooled to 45°C, mixed and the medium was inoculated with the sample in duplicate and allowed to solidify. Plates were incubated at 25˚C for 5 to 7 days.

RESULTS

Microbiological analyses

Table 2. Microbiological compliance results applied to the samples taken into the study

Purifying Oil Cleanser with %2 Postbiotics				
Analyze	**Result (cfu/g)**	**Limit for eye contour products and products for use in children under 3 years of age**	**Limit for other products**	**Evaluation**
Aerobic Mesophilic Colony Count	<1.0E+1	1.00E+02	1.00E+03	Suitable
P. aeruginosa	Not Detected	Must not be found	Must not be found	Suitable
Escherichia coli	Not Detected	Must not be found	Must not be found	Suitable
Candida albicans	Not Detected	Must not be found	Must not be found	Suitable
S. aureus	Not Detected	Must not be found	Must not be found	Suitable
Total Mould - Yeast Count	<1.0E+1	1.00E+02	1.00E+03	Suitable

Cell viability

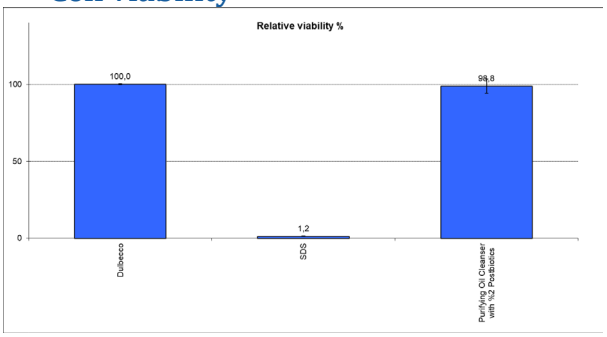

Figure 1. Cellular viability graphics.

According to the graphical data, the cleanser product containing 2% postbiotic did not show any cytotoxic effect on keratinocytes.

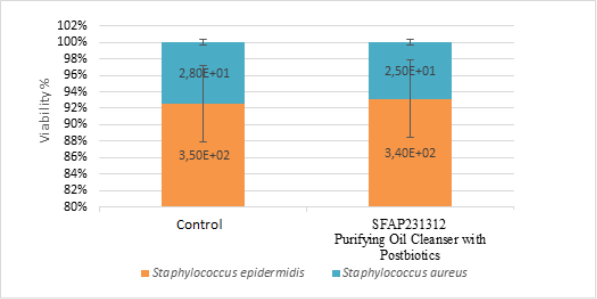

Figure 2. Pathogen non-pathogen balance graph of 2% postbiotic containing cleanser product in microbiota simulation test.

Microbiome & Microbiota Friendly Studies

It was hypothesised that the equilibrium test would demonstrate a preponderance of *S. epidermidis*. As this was observed, the study was continued. *S. epidermidis* is the indicator microorganism that suppresses the excessive increase of *S. aureus* species. The maintenance of a balanced microbiota is key to the suppression of infection. According to the graph, the tested product did not disturb the balance in favour of the pathogen.

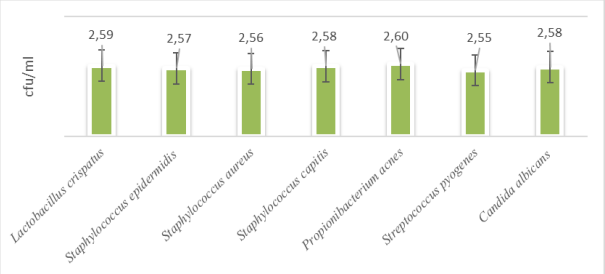

Figure 3. Plot of microbial diversity in the absence of samples.

The graph indicates that the initial diversity was determined in the analysis conducted without the addition of the tested product.

Table 3. Table of microbiological diversity results in absence of sample (control)

Microorganism	Control (PBS) cfu/ml	Control (PBS) log10
Lactobacillus crispatus	3.88E+02	2.59
Staphylococcus epidermidis	3.68E+02	2.57
Staphylococcus aureus	3.66E+02	2.56
Staphylococcus capitis	3.84E+02	2.58
Propionibacterium acnes	4.00E+02	2.60
Streptococcus pyogenes	3.55E+02	2.55
Candida albicans	3.78E+02	2.58

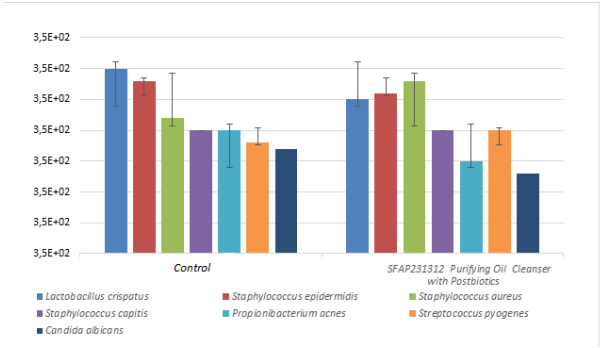

Figure 4. Microbial diversity graph of Purifying oil cleanser product containing 2% postbiotic.

The results of the study, as presented in the graph, indicate that the product containing 2% postbiotic did not result in any adverse effects on the diversity of microbiota.

Table 4. Table of microbiological diversity results in the presence of sample

Microorganism	Inoculum (cfu/ml)	Control (cfu/ml)	SFAP231312- Purifying Oil Cleanser with Postbiotics (cfu/ml)	Control (log10)	SFAP231312- Purifying Oil Cleanser with Postbiotics (log10)
Lactobacillus crispatus	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Staphylococcus epidermidis	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Staphylococcus aureus	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Staphylococcus capitis	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Propionibacterium acnes	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Streptococcus pyogenes	3.50E+02	3.5E+02	3.5E+02	2.5	2.5
Candida albicans	3.50E+02	3.5E+02	3.5E+02	2.5	2.5

The incorporation of the sample did not result in any impairment to the microbiological diversity. The study evaluated the change in microorganisms contacted with the sample in comparison to the control. The results demonstrated that the samples exhibited

no biocidal effect on microorganisms. The study's hypothesis was based on the principle that the difference between the initial amount and the amount after contact would not exceed 60%.

DISCUSSION

In recent years, there has been a marked shift in emphasis towards personalised medicine and preventive healthcare. This has resulted in a significant increase in consumer interest in natural-based cosmetics and personal care products. In the context of this paradigm shift, the importance of the skin microbiota is increasingly recognised due to its central role in mediating and regulating skin homeostasis (29). In conventional wisdom, the employment of live microorganisms has been a staple approach to promoting dermal health. However, the formulation and stability of these microorganisms can pose substantial challenges. Recent research has garnered mounting attention on the potential benefits of postbiotics, defined as metabolic byproducts secreted by microorganisms, particularly probiotic bacteria (30). Postbiotics are increasingly being recognised as promising ingredients for topical cosmetic formulations. They offer several advantages, including stability, safety and the ability to penetrate the skin barrier. Postbiotics have been shown to help maintain the body's homeostasis and aid in cellular repair. Furthermore, postbiotics have been demonstrated to enable microorganisms to function better (31). This research study investigates the safety and potential benefits of a new oil-based cleanser formulation, which contains a postbiotic in oil-soluble form as its unique feature. The results demonstrate that the formulation does not harm human keratinocytes and maintains a robust balance between pathogenic and commensal species in an in *vitro* skin microbiota simulation. Furthermore, cell viability tests show that the formulation does not cause significant adverse effects on the viability of human keratinocytes. This finding suggests that the formulation does not possess intrinsic toxicity, which is a significant indication in the field. It is particularly noteworthy that the majority of cosmetic products, notably cleansing formulations, are intended for long-term or frequent use. Therefore, it is imperative to preserve the integrity of keratinocytes, as elevated levels of toxicity can result in the disruption of skin barrier function, inflammation, and adverse reactions.In this context, the formulation appears to be safe for maintaining the health of epidermal cells.

The present study demonstrated that the ratio between non-pathogenic and pathogenic bacteria in an *in vitro* skin microbiota simulation, a community found in human skin, remained unaltered without compromising the balance. A healthy skin microbiota has been shown to assist in resisting pathogen colonization of human skin, modulating immune function, and maintaining the skin barrier. Numerous skin diseases are characterised by microbiota imbalance or overgrowth of certain pathogenic species, in contrast to the relative abundance associated with healthy microbiota.

The formulation that was the subject of this investigation has been shown to preserve microbial balance and to promote a healthy microbiota profile. This suggests that it has the potential to provide effective yet gentle cleansing without disrupting skin

barrier integrity or homeostasis.The benefits that were observed – specifically, the stimulation of beneficial species and suppression of pathogenic growth – are likely to be mediated by the inclusion of the postbiotic LTO 35, a metabolite derived from *Streptococcus thermophilus.* Postbiotics, defined as bioactive compounds produced by microbial fermentation (e.g., cell-free supernatant, enzymes, or organic acids), are increasingly recognised for their dermatological applications.In this context, the S. thermophilus-derived postbiotic contains a synergistic blend of metabolites, including lactic acid, bacteriocins, short-chain fatty acids (SCFAs), and hydrolytic enzymes, each contributing to skin health through distinct mechanisms: Lactic acid and organic acids lower cutaneous pH, creating an inhospitable environment for pathogens while enhancing ceramide synthesis, which reinforces stratum corneum cohesion. Bacteriocins exhibit dual functionality: (i) direct antimicrobial activity against pathogens (e.g., *Staphylococcus aureus*) and (ii) immunomodulatory effects via interactions with keratinocyte toll-like receptors. SCFAs (e.g., acetate, propionate) regulate epidermal differentiation and attenuate inflammatory cascades by modulating dendritic cell signalling (32).

The present findings are consistent with the evidence that postbiotic metabolites derived from probiotic strains, in particular lactic acid bacteria, exert pleiotropic benefits on cutaneous health without compromising commensal microbiota diversity (33). The formulation's selectivity – in other words, its capacity to inhibit pathogens while fostering symbionts – underscores its potential as a topical agent that is compatible with the microbiome.

CONCLUSION

The study demonstrated that the purifying oil cleanser, containing 2% postbiotic LTO 35, preserves the diversity of the skin microbiota, does not cause pathogenic dominance, does not damage keratinocyte cells and promotes cellular repair. Furthermore, microbiological balance tests revealed that the product has microbiome-friendly properties while protecting the skin barrier. These findings suggest that postbiotic-based formulations may play an effective role in protective dermocosmetic products.

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