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## KEY POINTS

- Existing dandruff treatments typically focus on antifungal effects although dandruff is a multicausal condition.
- Here, dandruff mechanisms and treatments are discussed, and an active based on olive leaf, jujube and levan is tested for its multifunctional effects against *M. furfur*, dandruff flakes and sebum.



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# Solving the Dandruff Dilemma Holistically and Naturally

Peer-reviewed



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**S**eborrheic dermatitis (SD) and dandruff are often considered the same chronic dermatological condition, although the former affects multiple seborrheic areas of the body such as the face, chest and scalp, whereas the latter is restricted to the scalp.<sup>1</sup> The symptoms associated with such conditions are often uncomfortable and can include irritation, itching and increased scaling of the scalp; it is still heavily debated whether inflammation symptoms such as red rashes and pruritus are specific to SD or if they can also occur in dandruff. In terms of scaling and flakes, these can be







## Immunodeficiency or deteriorated barrier integrity have been linked to the increased occurrence of dandruff.

dry or greasy and are in fact clustered corneocytes detaching from the stratum corneum.

Dandruff is a major concern of our time as it is widespread, with approximately 50% of the adult population affected, and it does not discriminate—every gender in every corner of the world experiences it.<sup>3</sup> The psychological impact of both SD and dandruff should not be underestimated. Those afflicted often suffer feelings of shame, embarrassment and loss of self-confidence, mainly due to itching in public and the appearance of flakes or scaling, which are falsely linked to a lack of hygiene.<sup>1,2</sup>

### A Head-scratcher

Even though dandruff is a highly discussed and researched dermocosmetic subject, many questions subsist. Defining a unique cause for its occurrence has not been possible, as its etiology is thought to be a multifactorial process, i.e., factors playing interdependent roles.

However, a link between dandruff and increased scalp colonization by the lipophilic *Malassezia* yeast genus has been established by multiple studies. While colonization of the skin by fungi is nothing abnormal, and *Malassezia* yeasts are part of the commensal human flora, compared with those who are not affected, the development of *Malassezia* yeasts is strongly increased.<sup>5,6</sup>

It has been determined that different species of *Malassezia* are involved in the development of dandruff: *M. globosa*, *M. restricta*, *M. dermatis* and *M. furfur*.<sup>6</sup> The seborrheic nature of the scalp makes it an ideal site for the colonization of such yeasts to feed on oils. The enzymes responsible for breaking up the sebum only hydrolyze saturated fatty acids (triglycerides), sparing some proinflammatory unsaturated

fatty acids. This leads to various types of scalp damage, which impairs the epidermal barrier.

The triggered inflammatory response that follows is what causes the itching associated with dandruff. As the body tries to repair the damage, a higher cell turnover occurs, ultimately manifesting as the shedding of visible flakes.<sup>7,8</sup> Increased sebaceous gland activity and the related higher sebum production can thus be correlated to an increased occurrence of dandruff. And, as mentioned, *Malassezia* feed on the scalp sebum, so with more sebum, more *Malassezia* colonization can be expected.<sup>7,8</sup>

The development of this condition is heavily dependent upon the host's predisposition, which consists primarily of the overall health of the scalp and the inflammatory response of the individual. In fact, immunodeficiency or disturbances such as a deficient stratum corneum and deteriorated barrier integrity have been linked to the increased occurrence of dandruff.<sup>4,6,7</sup>

Finally, environmental fluctuations such as sun exposure and cold weather have been found to exacerbate the appearance and severity of dandruff. Such extrinsic parameters could lead to skin dryness, which is known to worsen the condition.<sup>5</sup> Further aggravating factors can include lifestyle choices such as a poor diet, stress levels, and drug or alcohol consumption.<sup>2,7</sup>

### Itching for Solutions

Dandruff can be treated by various antifungals such as zinc pyrithione, which is considered to be one of the most effective. This active has extremely powerful antifungal and anti-dandruff activities and is therefore able to kill *Malassezia* yeasts. However, it has been added to the European list of prohibited cosmetic ingredients due to concerns over reproductive toxicity. This ban started on March 1, 2022, giving rise to the need for efficient alternatives for anti-dandruff formulations.<sup>9</sup>

Most existing anti-dandruff treatments focus mainly on an anti-fungal function. Nevertheless, it has been established that dandruff is

The dandruff treatment market is expected to expand at a CAGR of 5.8% from 2021 to 2028.

Source: Data Bridge Market Research



● Table 1. Commercial Shampoo Formulations Tested

a multicausal condition. Therefore, activities such as the restoration of the epidermal barrier function and anti-inflammatory and soothing effects should also be major targets for the prevention and treatment of dandruff.

Considering this approach, a natural cosmetic active containing olive leaf and jujube extracts and the moisturizing polysaccharide levan was screened for anti-dandruff capabilities. Olive leaf holds high oleuropein content, which has antioxidant properties. Jujube has previously shown skin-revitalizing and strengthening activities, while levan has demonstrated irritation-soothing activity. Previous studies, described elsewhere,<sup>10</sup> showed the combined active could improve cell renewal, strengthen cell resistance and activate proteasomes. Here, the active was tested in vitro for its effects

European cohort (mild and neutral pH)	Indian cohort (extra mild)
Water ( <i>Aqua</i> )	Water ( <i>Aqua</i> )
Sodium Laureth Sulfate	Cocamidopropyl Betaine
Sodium Chloride	Sodium Cocoyl Isethionate
Coco-Betaine	Lauryl Glucoside
Lauroyl/Myristoyl Methyl Glucamide	Peg-80 Sorbitan Laurate
Glycerin	Glycerin
PEG-4 Distearyl Ether	Citric Acid
Distearyl Ether	Sodium Benzoate
Dicaprylyl Ether	PEG-150 Distearate
Allantoin	Sodium Methyl Cocoyl Taurate
Kaolin	Fragrance
Guar Hydroxypropyltrimonium Chloride	Polyquaternium-10
Citric Acid	Disodium EDTA
Propylene Glycol	
Parfum	
Sodium Benzoate	

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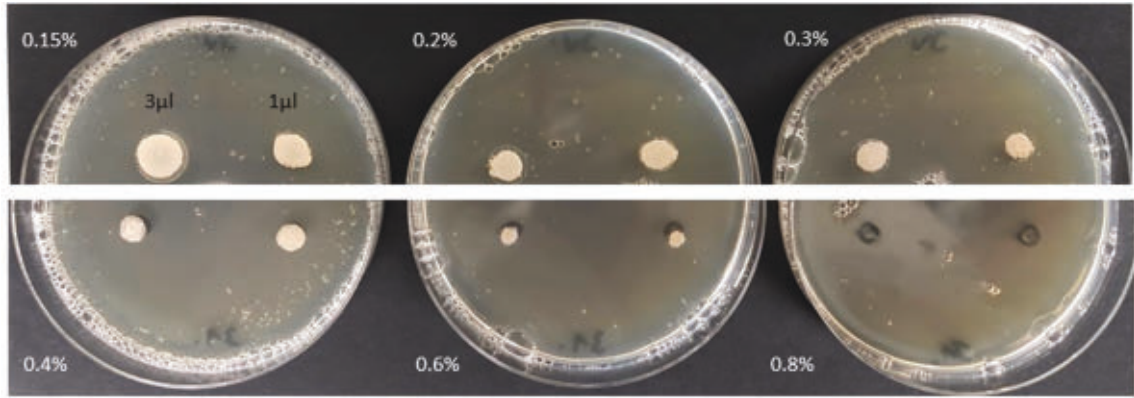
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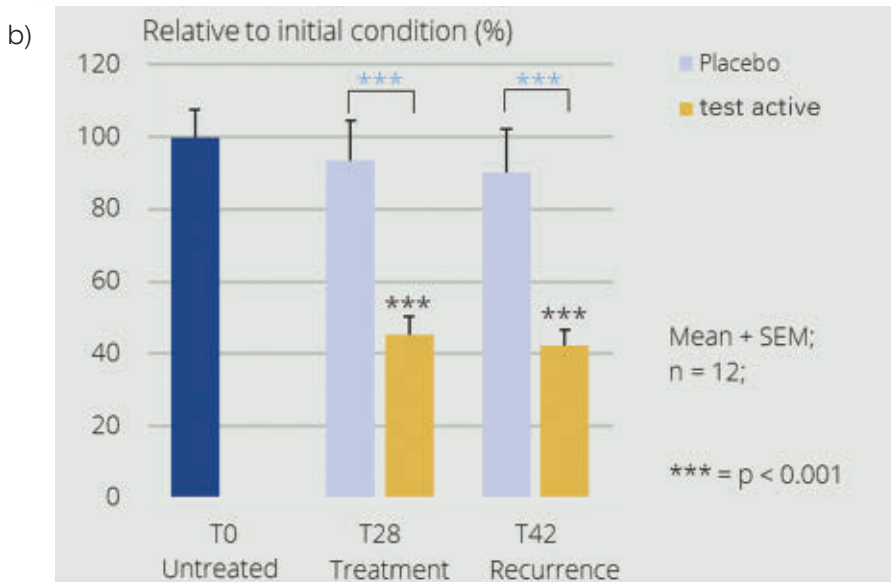
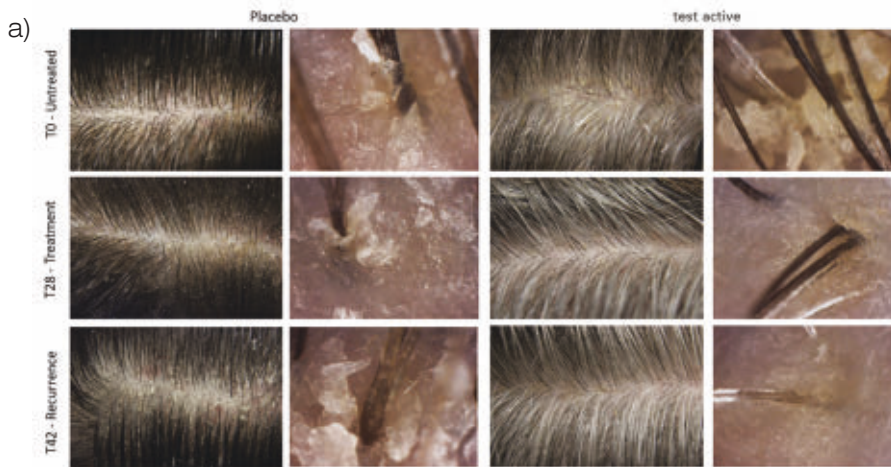
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Agar plates with different test active<sup>a</sup> concentrations inoculated with different volumes of *M. furfur* strain; results of the active at 0%, 1%, 1.2% and 1.6% are not shown

● Figure 1. Minimal inhibitory concentration of *M. furfur*



● Figure 2. Dandruff reduction in European subjects after treatment with 2% test active vs. placebo at different time points

on *Malassezia furfur* and in vivo for the elimination and prevention of dandruff in a rinse-off study of European and Indian subjects.

### In vitro Materials and Methods

**Experimental design:** Different concentrations of the test active<sup>a</sup> were used in two different test shampoos in both European and Indian subjects, the majority of whom suffered from moderate to heavy scalp scaling; a clinical score of heavy dandruff was not included. The volunteers also had not used anti-dandruff agents prior to the study.

**In vitro antimicrobial effects:** Previously, in a preliminary assay (not shown), a screen-

<sup>a</sup> *Proteolea* (INCI: Glycerin (and) Water (Aqua) (and) Levan (and) Decyl Glucoside (and) Olea Europaea Leaf Extract (and) Phenethyl Alcohol (and) Ziziphus Jujuba Seed Extract (and) Citric Acid (and) Ascorbic Acid) is a product of Rahm AG.



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## Dandruff generally did not reappear in hair treated with the test active; in cases where it did, the severity was significantly less.

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ing test following the Kirby-Bauer method was performed with multiple actives; 0.1 mL of each active at a 10% concentration was applied on agar plates containing Müller-Hinton medium and an emulsion with the *M. furfur*. Inhibition zones were then determined.

Based on promising results from this work, the minimal inhibitory concentration (MIC) of the test active against *M. furfur* (ATCC 14521) was measured as described here in triplicate. The zone of inhibition with 10% of the ingredient was performed according to a slightly modified Kirby-Bauer method.<sup>11</sup> Briefly, *M. furfur* was cultivated in tryptone-soya bouillon containing olive oil at 30-35°C for 24-48 hr. The test strain was applied to Müller-Hinton agar plates (10 mm, 0.1 mL, 30-35°C/72 hr), after which the MIC was determined using the agar

dilution procedure performed according to J.H. Jorgensen und M.A. Pfaller.<sup>12</sup> Müller-Hinton agar plates with nine different concentrations of the test active, ranging from 0.1-1.6%, and a placebo without the active were produced. *M. furfur* (ATCC 14521) was inoculated (3 µL and 1µL) and incubated for three days at 30-35°C.

### In vivo Methods

A two-center double-blind study was conducted to determine the anti-dandruff efficacy of the test active<sup>a</sup> in terms of elimination and reappearance. The study was designed in compliance with the regulation Guidelines for Research on Human Beings and guidelines of the Scientific Committee on Consumer Safety (SCCS).

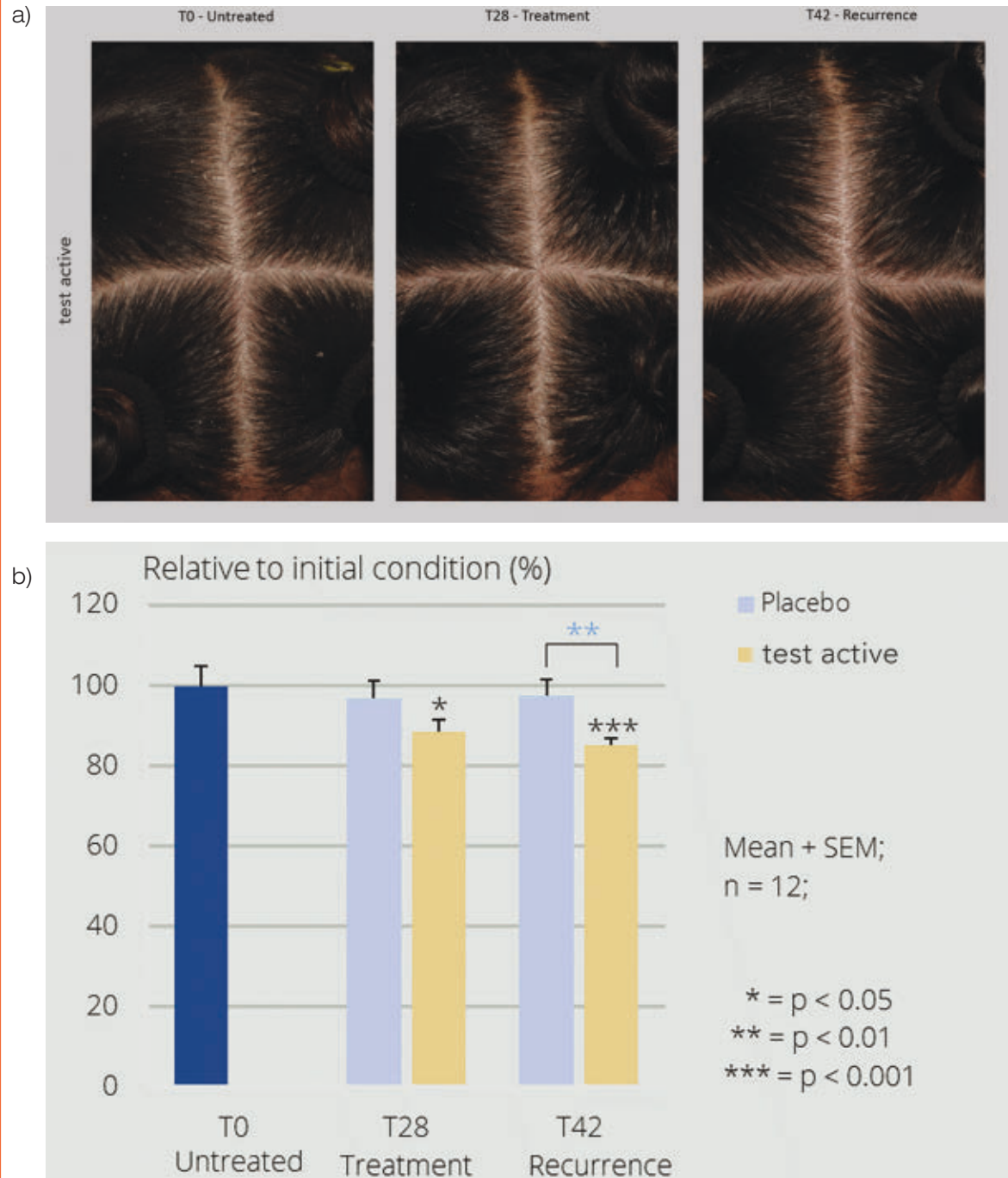
**Volunteers and conditions:** In total, 48 volunteers with moderate to severe dandruff



were enrolled, including 12 for each condition. The test groups included 12 Indian women ages 18-35, and six male and six female Europeans, ages 22-60. These subjects washed their hair with a shampoo containing 0.5% (Indian volun-

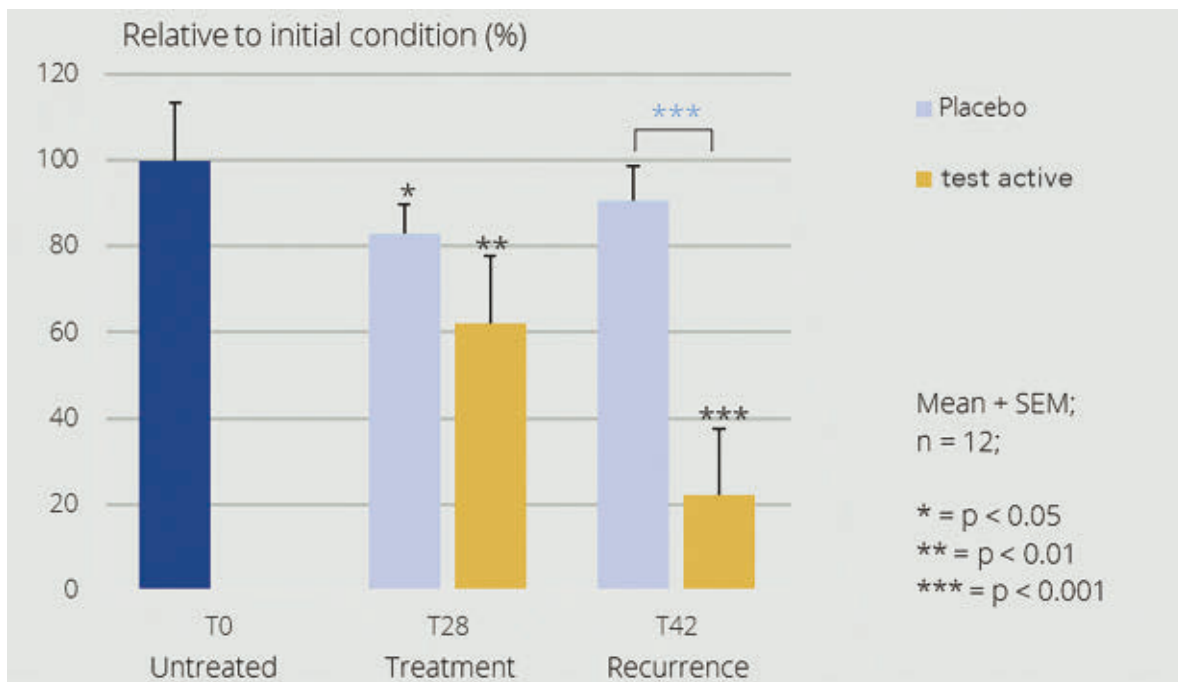
teers) or 2.0% (European volunteers) of the test active every second day for 28 days.

The placebo groups also included six female and six male Europeans, ages 21-54; and 12 Indian women, ages 18-35 years. They followed



● Figure 3. Dandruff reduction in Indian subjects after treatment with 0.5% test active at different time points





● Figure 4. Sebum measurements for Indian cohort at different time points

the same experimental setup using a shampoo without the test active for 28 days. Finally, to evaluate the reappearance of dandruff, the following 14 days after treatment, all volunteers used the placebo shampoo without the active ingredient.

**Test formulas:** In terms of the tested formulations, commercial neutral and mild shampoos were purchased, to which the test active was added in the specified concentrations (0.5% and 2%). For the European cohort, a mild and pH neutral shampoo was used; in the Indian cohort, an extra mild shampoo was used (see **Table 1**).

**Visual evaluation:** The volunteers' scalps and hair were photographed and evaluated by specialized technicians. Self-evaluations by panelists also were performed.

**Sebum measurements:** Sebum measurements were taken using a sebumeter<sup>b</sup> controlled conditions. For the Indian measurements were made on subjects' foreheads near the hairline, and for European cohort, directly on the sc

<sup>b</sup> Sebumeter 815, Courage & Khazaka

**Statistics:** Statistical values were determined with the paired or unpaired Student's *t*-test, depending on the condition. In the results section, the values in black are the result of comparison with the baseline (paired), and in blue are the result of comparison with the placebo (unpaired).

## In vitro Results

Agar plates with the test active<sup>a</sup> at different concentrations showed total visible growth inhibition of *M. furfur* at a concentration of 0.8% (volume/volume).





This experiment also showed weakened fungi growth at a 0.2% concentration, with an IC50 of approximately 0.4% (see **Figure 1**).

## In vivo Results

### **Dandruff results, European cohort:**

Treatment with 2% of the test active after 28 days resulted in a highly significant dandruff reduction of up to 54% (see **Figure 2b**) in the European group. In comparison, the placebo treatment showed an average reduction of only 6%. **Figure 2a** shows representative pictures of scalp areas treated with the placebo and 2% test active at different time points.

As noted, to evaluate the reappearance of dandruff, all volunteers used the placebo shampoo without the test active for 14 days following the initial 28-day treatment (T42-Recurrence). Results showed that dandruff generally did not reappear in hair treated with the test active, and in cases where it did, the severity was significantly less than at the beginning of the study. The results in comparison with the placebo were highly significant as well. Quantification of the data is shown in **Figure 2b**.

In terms of self-evaluation, after 28 days of using the shampoo with 2% test active, in the European cohort, 91.7% of the volunteers felt their condition improved after treatment. Furthermore, at day 42, after two additional weeks of using the placebo shampoo without the test active, 100% of volunteers had less dandruff and only 8.3% reported a slight dandruff reappearance (data not shown).

**Dandruff results, Indian cohort:** In the Indian group, treatment with 0.5% of the test active for 28 days reduced dandruff by 11% (see **Figure 3a**). Notably, after 28 days, this difference was only significant compared with the initial control but after 42 days, it also was significant compared with the placebo; in contrast, the placebo treatment, on average, reduced dandruff by 3%. **Figure 3a** shows representative pictures of the scalp for treatment with 0.5% test active at different time points.

While the difference between days 28 and 42 was not large, after 42 days, the standard deviation became smaller and therefore significant. The results indicated the anti-dandruff efficacy was dose- and time-dependent.

Once again, to evaluate the reappearance of dandruff, for the subsequent 14 days after the

initial phase of the study, all volunteers continued to use the placebo shampoo without the active ingredient. Results showed that dandruff generally did not reappear and that in cases where it did, the severity was much less than at the beginning of the study.

In terms of self-evaluation, 100% of Indian volunteers reported an improvement after treatment with the test active. Interestingly, 55% of volunteers also noted an improvement as early as after the third washing; 45% also reported having softer hair. Dermatological assessments revealed a significant reduction in pruritus and itching in the test group; this was not observed in the placebo group (data not shown).

### **Sebum measurements, Indian cohort:**

**Figure 4** shows the results of the sebumeter measurements for the Indian cohort. A significant reduction in sebum was observed after 28 days of using the test shampoo, compared with the initial condition (T0); namely a reduction of 17% for the placebo and 38% for the test active. On day 42, in the case of the placebo group, sebum levels appeared to re-establish themselves, returning to 91% of the original sebum levels. In the case of the test group, however, sebum levels decreased even further, down to 22% of the original levels.

This unexpected further decrease amounted to a nearly 78% reduction in total and was highly significant in comparison with the initial control and placebo. Thus, the sebum values in the test group were lowered from 189  $\mu\text{g}/\text{cm}^2$  to 42  $\mu\text{g}/\text{cm}^2$  (normal scalp range = 33–66  $\mu\text{g}/\text{cm}^2$ ), while the placebo stayed in the range of an oily scalp (T0 – 173  $\mu\text{g}/\text{cm}^2$ ; T42 – 157  $\mu\text{g}/\text{cm}^2$ ; oily scalp range > 66  $\mu\text{g}/\text{cm}^2$ ).

### **Sebum measurements, European cohort:**

The sebum measurements in the European cohort were inconsistent. Here, the measurements were performed directly on the scalp, and this caused high variations in the values obtained. Nevertheless, a similar tendency for the test formulation was observed—the sebum values were reduced consistently, reaching a total reduction of 30% after 42 days.

## Discussion and Conclusions

In vitro, increasing concentrations of the test active showed distinct reductions in *M. furfur* colonization, leading to a complete inability of the yeast to grow in the presence of 0.8% active. As noted, *Malassezia* fungi play



a central role in dermatological disorders such as dandruff, and previous studies show a drastic reduction of dandruff symptoms when the development of *Malassezia* fungi is inhibited. Therefore, these in vitro findings support the anti-fungal activity of test active and its use for anti-dandruff-oriented products.

In vivo, a test active concentration as low as 0.5% incorporated in a shampoo was shown to be effective against dandruff; at 2%, it demonstrated further pronounced reductions in symptoms. While the precise mechanism of action is unknown, the combination of the ingredient's antioxidant activity, skin revitalizing and strengthening activities, and soothing effects could be providing the anti-dandruff efficacy observed.

The results obtained through dermatological evaluations were in line with the results of the self-evaluations. The participants observed a clear alleviation of flaking and itchiness. Overall, they also felt better, experienced less scratching and presented with a healthier scalp.

The in vitro and in vivo studies presented here show that the natural test active<sup>a</sup> inhibited the proliferation of *M. furfur* and reduced dandruff symptoms such as flaking and itching. Taken together with previous work, which showed the active promotes stress resistance in cells, improves skin renewal and activates proteasomes,<sup>10</sup> the test active is recommended for the holistic treatment of dandruff to alleviate symptoms, fight the root causes and promote the scalp's return to a healthy state.

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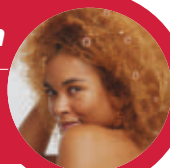
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