TRIKENOL PLUS

Dandruff is a scalp problem that is manifested by excessive desquamation, forming aggregates of corneal cells as a result of acceleration in the renewal of epidermal cells. TRIKENOL PLUS is a new complex of natural origin active ingredients for the treatment of dandruff.

SCALP AND DANDRUFF

The scalp is an area with a high number of hair follicles, sebaceous glands and sweat glands, and a vast and complex innervation and vasculature. The sebaceous glands attached to hair follicles secrete sebum, which is deposited on the scalp and provides it with protection and lubrication, being the main responsible for hair flexibility and brightness.

Dandruff is defined as excessive desquamation of the scalp accompanied by mild itching without signs of inflammation. The appearance of dandruff is the result of an increase in the rate of desquamation of the epidermis, where cells of the basal layer require half the time needed in a normal scalp to reach the stratum corneum. This rapid proliferation causes an increase in the number of corneal cells, accompanied by an anomalous cementation and the appearance of large aggregates of corneal cells (Hay RJ, 1997).

Types of dandruff

1. Dry form or Pityriasis simplex

It is the most common form. People who suffer it have a dry, lackluster and rough scalp. The scales are thin, white-gray in color, more or less adherent, and they can be detached after combing or spontaneously. No inflammatory signs are present.

2. Seborrheic form or Pityriasis steatoides

It is a desquamation state associated with the existence of seborrhea. The hair has a greasy and shiny appearance, with yellowish and thicker scales. It can be accompanied by erythema, with more or less intense itching, and inflammation of the scalp. It can also cause hair loss.
Triggering mechanisms

A number of factors influence this functional disorder:

- **Environmental conditions.** Dandruff manifests more acutely in winter and decreases in summer.

- **Hyperproliferative activity of the epidermis.** There is an increase in mitotic activity of cells in the basal layer of the epidermis of the scalp, so that the path of the keratinocytes to the stratum corneum takes half the time (14 days).

- **Hormonal imbalance.** Dandruff usually appears at puberty and progressively increases until the age of 30.

- **Microbial flora.** The scalp is colonized by microorganisms with a density of around $10^3$-$10^5$ microorganisms/mm$^2$, formed by species of *Staphylococcus*, *Propionibacterium* and *Malassezia*. Due to the appearance of dandruff, the relative proportion of *Malassezia* is multiplied by 1.5-2.0, up to 75% of existing flora. The presence of excess fat promotes the multiplication and development of this microorganism, because it can degrade sebum triglycerides. This leads to an increase in the amount of free fatty acids existing in the scalp, especially oleic acid, which has a strong irritating ability that can cause the stimulation of mitosis in cells of the basal layer.

**Malassezia**

The *Malassezia* genus comprises lipophilic yeasts differentiated in two species with defined physiological properties: lipophilic strains such as *Malassezia pachydermatis* able to grow on routine laboratory media and lipophilic yeasts that require the addition of long-chain fatty acids to the culture medium for their development, such as the *Malassezia furfur (=Pityrosporum ovale)* species.

Recent studies on their characteristics have led to the classification of lipid-dependent strains in six genetically distinct species: *M. furfur (=Pityrosporum ovale)*, *M. globosa*, *M. sympodialis*, *M. obtusa*, *M. restricta* and *M. slooffiae* (Aspiroz MC et al., 1997).

Other recent studies on dandruff show that the species of *Malassezia* detected in the scalp have been *M. restricta* and *M. globosa*, while *M. furfur (=P. ovale)* has not been detected and therefore could be ruled out as a potential dandruff agent (Gemmer et al., 2002)

Another study indicates that *M. restricta*, *M. globosa* and rarely *M. sympodialis*, have been detected in the scalp, but not *M. furfur (=P. ovale)*, *M. obtusa*, *M. slooffiae* and *M. pachydermatis*. It is explained that the mechanism causing the dandruff is the presence of *Malassezia globosa*, which hydrolyzes sebum triglycerides from the scalp using its lipase, resulting in the release of oleic acid. This irritating fatty acid penetrates the stratum corneum of the scalp breaking the barrier function, triggering the hyperproliferation of the scalp and
inducing a greater sebum secretion, so that it continues to feed the population of *Malassezia* (EHRS Conference, 2002).

**Anti-dandruff treatment**

Current anti-dandruff formulations usually contain one or more active ingredients to correct each one of the causes, such as:

- **Anti-fungal ingredients**:azole family (climbazole).
- **Germicides**: undecylenic acid, hexachlorophene, ketoconazole, quaternary ammonium compounds, etc.
- **Cytostatic action germicides**: sulfur, selenium, zinc pyrithione, piroctone olamine, etc.
- **Anti-seborrhic ingredients**: sulfur compounds, some plant extracts.
- **Keratolytic ingredients**: α-hydroxy acids, salicylic acid, selenium sulfide, sulfur derivatives, urea.
- **Anti-itch ingredients**: panthenol, some plant derivatives.

In all cases, effectiveness depends on the type of cosmetic formulation and the contact time of the active principle with the scalp.

**COMPOSITION**

**TRIKENOL PLUS** is a synergistic blend of two plant origin ingredients, *terpinen-4-ol* and *willow extract*, enriched with *salicylic derivatives* and developed to provide an effective anti-dandruff active ingredient obtained from natural sources.

**Terpinen-4-ol**

Terpinen-4-ol is obtained by fractional distillation of tea tree oil (*Melaleuca alternifolia*). Its color varies from transparent to pale yellow and it has a very characteristic odor mixture of earth, wood and pepper.
Efficacy

**Antimicrobial**

It has been evaluated to observe its action against a wide range of microorganisms:

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>MIC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>0.25</td>
</tr>
<tr>
<td>Coryneform spp.</td>
<td>0.01</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>0.10</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.25</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0.50</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>0.05</td>
</tr>
<tr>
<td>Pityrosporum ovales (M. furfur)</td>
<td>0.05</td>
</tr>
<tr>
<td>Tricophyton mentagrophytes</td>
<td>0.05</td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 1. Efficacy of Terpinen-4-ol against microorganisms

**Anti-inflammatory**

The anti-inflammatory efficacy was evaluated by studying the reduction of erythema, induced by ultraviolet radiation using solar simulation. Results show that, in addition to antimicrobial activity, terpinen-4-ol has an anti-inflammatory action (Hart PH, 2000).

The benefits of Terpinen-4-ol are its stability, broad spectrum activity and anti-inflammatory action.
Willow extract

Willow, *Salix alba*, is an evergreen tree belonging to the salicaceae family up to 25 m high, with an erect trunk and a dark gray bark with large grooves, ending in a narrow crown. Its leaves have a silvery-gray color and they are serrated, up to 10 cm in length and covered with soft hairs on both sides. The flowers arranged in catkins appear before the leaves.

The parts used in cosmetics are the dried leaves and bark of branches of *Salix alba*.

The main constituents are polyphenols, flavonoids and salicylic derivatives. Polyphenols (8-20%) are represented by catechic type tannins. Flavonoids (0.2-1.5%) are characterized by the glucosides of isoquercitrin, naringin, isosalipurposide, quercetin andisorhamnetin.

Salicylic derivatives (1.5-11%) are represented by salicin or salicoside, which is a glucoside in saligenin, salicortin, tremulacin and salireposide. It also contains organic acids (salicylic, vanillic, syringic, caffeic, p-hydroxybenzoic, coumaric and ferulic acid), as well as oligo- and polysaccharides (pectins, galactans and arabinans).

Willow extract is widely used in cosmetics and dermatology due to its moisturizing, keratolytic and purifying properties. It also has properties to be used as an astringent, analgesic, anti-inflammatory and antimicrobial active ingredient (Council of Europe, 2001).

In this case, it has been incorporated to TRIKENOL PLUS due to its anti-irritant, anti-seborrheic and keratolytic action.

Salicylic derivatives

One of the best known and most representative is salicylic acid (2-hydroxybenzoic acid), with the molecular formula C_7H_6O_3 and CAS number: 69-72-7. This substance is described in many pharmacopoeias (F. Eur, USP 25, F China, F Int., F. Japan, F Pol.).

Salicylic acid is applied topically to treat keratolytic and skin desquamation disorders.

Its keratolytic properties (Huber et al., 1977) are also widely demonstrated in the literature. The exfoliating action resulting from a decrease in cohesion between corneocytes and a normalization of the desquamation of
the stratum corneum is due to the inhibition of enzymes involved in the cementation of corneocytes (Béani JC, 2002).

**IN VITRO EFFICACY**

**Antifungal Activity**

The activity of TRIKENOL PLUS was studied against *Malassezia globosa* and *Malassezia furfur (=P. ovale)*, determining the values of:

- **Minimum Inhibitory Concentration (MIC):** minimum concentration that produces a total inhibition of growth.
- **Strong Inhibition (SI):** concentration that produces a considerable decrease in growth without causing total inhibition.

This information is of great interest, because the aim is not a total action by the active ingredient, but an inhibition which is enough so that skin’s defense mechanisms can act.

<table>
<thead>
<tr>
<th>Malassezia globosa</th>
<th>Malassezia furfur (=Pityrosporum ovale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC</td>
<td>SI</td>
</tr>
<tr>
<td>0.098%</td>
<td>0.024%</td>
</tr>
<tr>
<td>MIC</td>
<td>SI</td>
</tr>
<tr>
<td>0.195%</td>
<td>0.024%</td>
</tr>
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</table>

Table 2. Results of TRIKENOL PLUS in vitro test

TRIKENOL PLUS is active against the two Malassezias involved in dandruff, *M. furfur* and *M. globosa*, but it shows greater activity against *M. globosa*, which has a more relevant role in dandruff, according to the recent studies mentioned above.

**Anti-irritating Activity**

In order to evaluate the anti-irritant efficacy of the Willow Extract in TRIKENOL PLUS, the inhibition of cyclooxygenase (COX), enzyme that catalyzes the formation of inflammatory mediators from arachidonic acid, was measured.

The assay was performed on primary cultures of keratinocytes determining the content of prostaglandins (PGE2) using an immunoassay and acetylsalicylic acid as a positive control. The results of this enzymatic assay are shown in figure 5. It can be seen that Willow Extract (0.1%) produces an important reduction in the
amount of prostaglandin (PGE2), which leads to a marked inhibition of cyclooxygenase. This effect is comparable to those caused by acetylsalicylic acid used as control in the assay (0.1 mM).

Figure 5. Results of the anti-irritant action of willow extract.

COSMETIC APPLICATIONS

TRIKENOL PLUS is a new natural origin active ingredient for the treatment of dandruff and the various factors involved, both in dry dandruff and in seborrheic or greasy dandruff.

<table>
<thead>
<tr>
<th>ACTION</th>
<th>ACTIVE INGREDIENT</th>
<th>EFFICACY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial proliferation inhibitor</td>
<td>Set of active ingredients of TRIKENOL PLUS</td>
<td>MIC=0.098 (M. globosa) and 0.195 (M. furfur)</td>
</tr>
<tr>
<td>Anti-irritant</td>
<td>Willow extract</td>
<td>Cyclooxygenase inhibition=43.4%</td>
</tr>
</tbody>
</table>