



TRIKENOL™ PLUS

The natural alternative to treat acne-prone skin
and balance microbiota



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ACNE: NOT JUST A QUESTION OF AESTHETICS



Anyone who has suffered from any type of acne knows that it is a serious problem, due to the difficulty of getting rid of it.

Acne is an inflammatory disorder, which is very common in adolescents and produces papules, pustules, nodules and other forms of skin lesions. According to the study conducted by Collier et al., 42.5% of men and 50.9% of women between 20 and 29 years old suffer from this condition. It is the most common diagnosis observed in dermatological outpatient practices in the United States (Zeichener et al., 2017). According to Suh y Kwon (2015), acne is a virtually universal skin inflammatory disorder that affects more than 85% of adolescents worldwide.

Although acne has traditionally been associated with adolescents, a significant proportion of adults are affected (Collier et al. 2008), both women and men, although in women its effect is greater and prevails for a longer time (Zeichener et al., 2017). In fact, different epidemiological studies show that there is an increase in postadolescent acne and that, in those cases, the condition lasts longer and requires treatment until the mid-forties (Knaggs et al., 2004).

Acne also causes depression and anxiety (Uslu et al., 2008). Scars can be serious (Lacarrubba et al., 2013) and some lesions can leave permanent marks (Rao, 2011). Acne and associated depression have a greater effect on women than on men.

Trikenol™ Plus is a complex of natural origin active ingredients designed to treat acne-prone oily skin.

Trikenol™ Plus, treatment for acne-prone skin in both adolescents and adults.

WHY DOES ACNE APPEAR?



Acne originates in the pilosebaceous unit with hyperseborrhea. Follicular obstruction, at the beginning of the lesion, may occur due to hyperkeratinization of the follicular epithelium. Inflammation can be considered as the defining characteristic of acne.

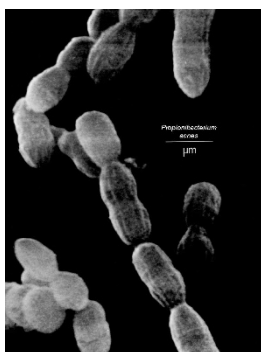
It is believed that four factors play a key role in the development of acne lesions (Gollnick, 2015):

- excessive sebum production.
- alteration of keratinization within the follicle.
- colonization of the pilosebaceous duct by microorganisms, such as *Cutibacterium acnes*.
- release of inflammatory mediators in the skin.

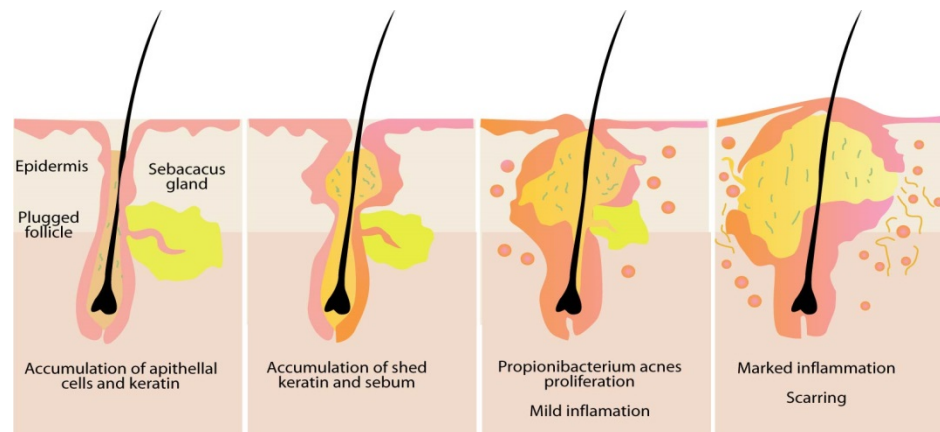
The accumulation of lipids and keratin allows the proliferation of anaerobic microorganisms.

Other factors, such as hormonal fluctuations, genetics, cosmetics, diet, tobacco use and stress have been linked to the appearance of acne in adult women.

The predominant microorganism in the follicular flora is *Cutibacterium acnes*. In addition, *Staphylococcus aureus*, yeasts, *Malassezia furfur* and *Malassezia globosa* have been isolated in many sebaceous follicles.



Cutibacterium acnes is an anaerobic species that grows in the sebaceous follicle and it is transported towards the skin surface by the flow of sebum (Nishijima S. et al., 2000). The obstruction of the pilosebaceous canal (hyperkeratinization) increases the intracomedo proliferation of *C.acnes*. This organism has lipase activity, which acts on triglycerides producing fatty acids that are irritating and comedogenic. Furthermore, this species has the ability to produce enzymes such as proteases, hyaluronidases and phosphatases, and to secrete chemotactic factors, which are involved in the inflammatory process (Leyden JJ., 2001).



An effective anti-acne product must meet the following requirements:

- Reduce sebum production.
- Unblock pilosebaceous canals.
- Reduce the responsible microbial flora.
- Reduce irritation.

The use of botanical and phytochemical products in dermatological products is increasingly popular and many patients resort to these therapies for the treatment of acne (Whitney et al., 2014), as an alternative to other more aggressive options with unwanted side effects.

Trikenol™ Plus is presented as the natural solution to the treatment of acne.

Trikenol™ Plus, nature's wisdom to fight acne.

TRIKENOL™ PLUS AS A SOLUTION TO ACNE



Trikenol™ Plus is a synergistic blend of two plant origin ingredients, terpinen-4-ol and willow extract, enriched with salicylic derivatives to provide natural anti-acne activity.

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 characteristic odor mixture of earth, wood and pepper. It has anti-inflammatory and antimicrobial activity (Pazyar et al., 2013).

Willow extract, obtained from *Salix alba*, is widely used in cosmetics and dermatology due to its moisturizing, keratolytic, anti-irritant and antiseborrhoeic properties. It also has properties to be used as an astringent, analgesic, anti-inflammatory and antimicrobial active ingredient (Council of Europe, 2001). It is rich in polyphenols, flavonoids and salicylic derivatives.

One of the best known and most representative is **salicylic acid**. This substance is applied topically to treat keratolytic and skin desquamation disorders. 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

Antimicrobial activity

The in vitro antimicrobial activity of Trikenol™ Plus was studied against *Cutibacterium acnes*, *Staphylococcus aureus*, *Malassezia furfur* (=P. ovale) and *Malassezia globosa*, in the inflammatory processes of acne that occur in pilosebaceous follicles and the creation of the pore anaerobic conditions that promote pore inflammation.

To this end, the following values were determined:

-Minimum Inhibitory Concentration (MIC) (minimum concentration of substance to be tested that produces total inhibition of microorganism growth)

-Strong Inhibition (SI) (concentration of substance used to produce a considerable decrease in growth without causing total inhibition). This information is of great interest, because from a dermatological point of view, the aim is not a total action by the active ingredient, but an inhibition that produces a decline in microbial population which is enough so that the skin's own defense mechanisms can act.

<i>Cutibacterium acnes</i>		<i>Malassezia globosa</i>		<i>Malassezia furfur</i>		<i>Staphylococcus aureus</i>	
MIC	SI	MIC	SI	MIC	SI	MIC	SI
0.39%	0.39%	0.098%	0.024%	0.195%	0.024%	1.56%	0.78%

Results of the *in vitro* antimicrobial test.

The results obtained confirm that Trikenol™ Plus is a bactericidal and fungicidal active ingredient useful in the treatment of acne-prone oily skin. With more effective activity against *Malassezia*, it prevents it from hydrolyzing triglycerides from sebum.

At the same time, Trikenol™ Plus protects the beneficial microbiota and achieves a balance of the bacterial flora that results in healthy skin. The following results show how beneficial skin bacteria are not affected by the active.

<i>Lactobacillus plantarum</i>		<i>Streptococcus thermophilus</i>		<i>Bifidobacterium breve</i>	
MIC	SI	MIC	SI	MIC	SI
3,12%	3,12%	3,12%	3,12%	3,12%	3,12%

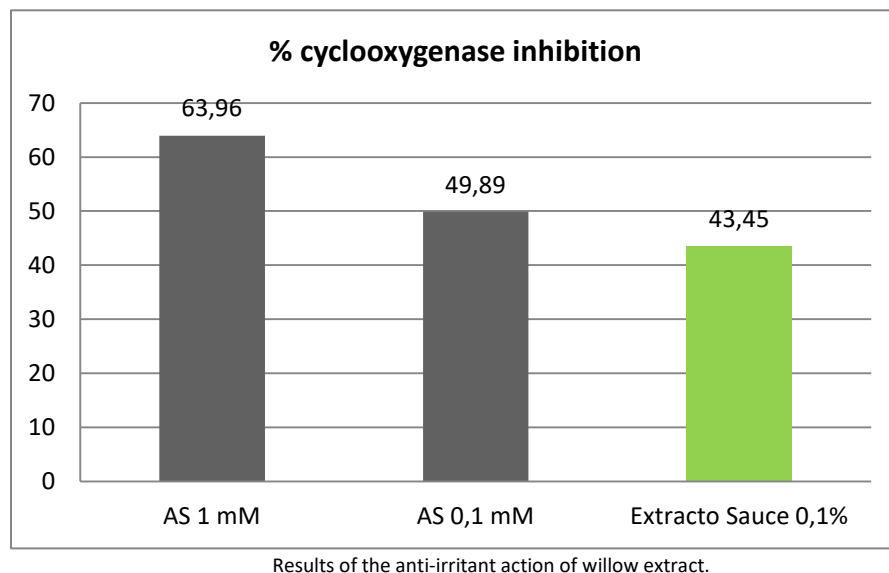
Trikenol™ Plus balances cutaneous microbiota.

Anti-irritating Activity

In order to evaluate the anti-irritant efficacy of 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 human keratinocytes by determining the content of prostaglandins (PGE2) using an immunoassay. The actual culture medium of keratinocytes was used as a negative control, and the acetylsalicylic acid (AS) was tested at concentrations of 1 mM and 0.1 mM as a positive control.

The following chart shows the values obtained in this assay. 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).



IN VIVO EFFICACY

An *in vivo* study was carried out to test the efficacy of Trikenol™ Plus against acne. This study was organized as follows:

- 64 volunteers, men and women, between 18 and 32 years old, with acne-prone skin.
- The panel was divided into 2 groups (32 volunteers each). One group was treated with a facial cleanser and a gel with the active ingredient at 0.8% and the other group was treated with a facial cleanser and a placebo gel.
- 42-day treatment.
- Daily face application.
- Measurements at D0, D21 and D42.

Effect in sebum reduction

One of the triggers in the development of acne-prone skin is excessive sebum production.

To determine the activity of sebaceous glands, a Sebufix® strip was placed on the forehead of the volunteers. This film absorbs skin sebum, which appears as black spots. The strips were then analyzed. The number of active sebaceous glands and the surface area (mm²) occupied by secreted sebum was calculated.

At day 21, Trikenol™ Plus **reduced sebum production by 77% and the number of active sebaceous glands by 32%**, versus placebo.



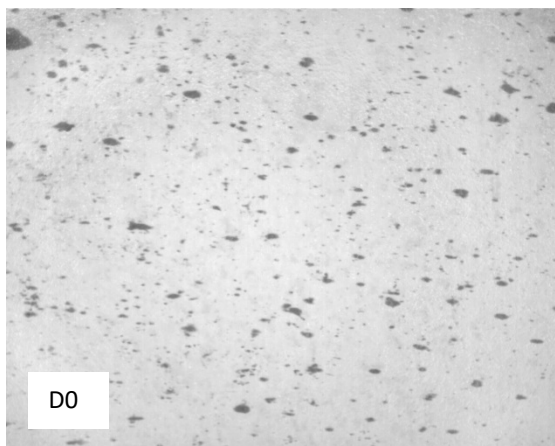
-77%

**Sebum
production
(vs placebo)**

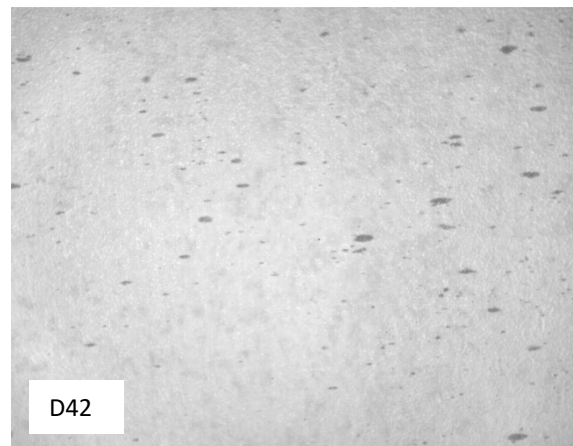


-32%

**Active sebaceous
glands
(vs placebo)**



D0



D42

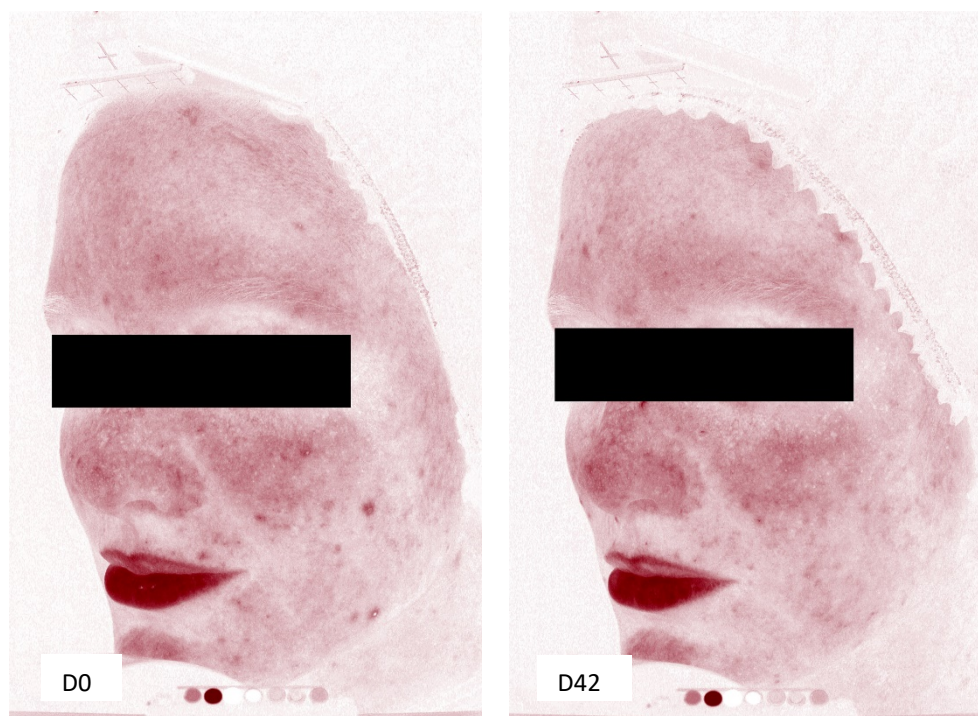
Sebufix+Visioscan images.

Reduction in acne lesions

On the one hand, the reduction in acne lesions was measured using VISIA-CR. On the other hand, a dermatologist counted the decrease in inflammatory and non-inflammatory lesions.

Trikenol™ Plus **reduced the area affected by inflammatory lesions by 12% at 21 days**, versus placebo.

↓ **-12%** Red dotted area
(vs placebo)

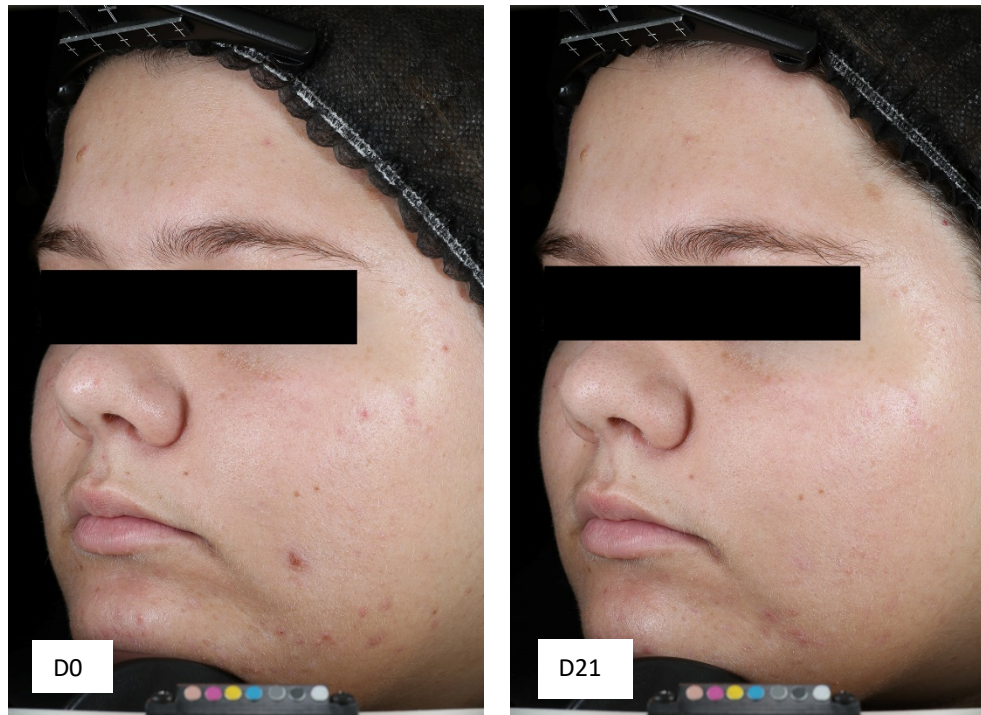


VISIA-Red Images.

Moreover, at 21 days, Trikenol™ Plus **reduced inflammatory lesions by 5.2% and non-inflammatory lesions by 25.4%.**

↓ **-5.2%** Number of
inflammatory
lesions
(vs placebo)

↓ **-25.4%** Number of non-
inflammatory
lesions
(vs placebo)



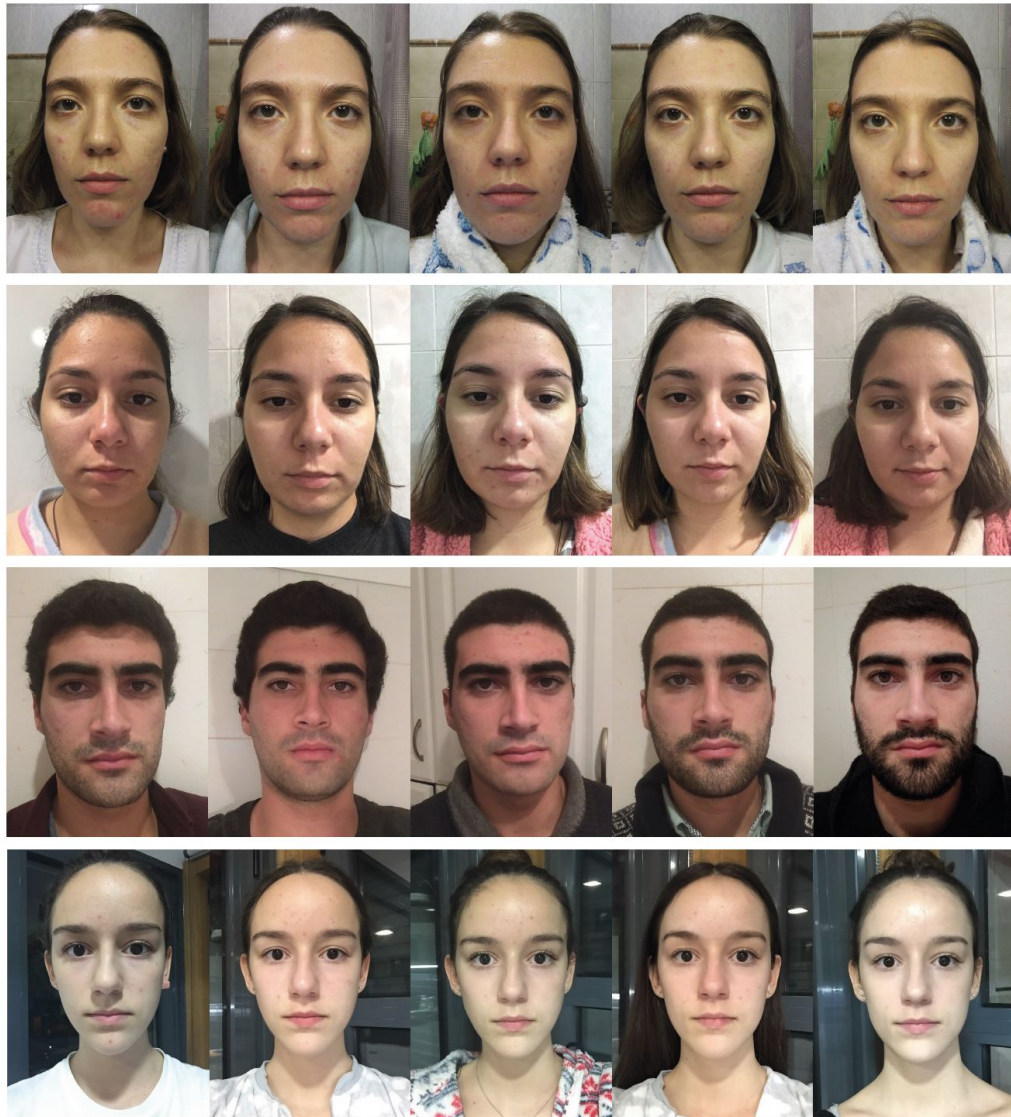
VISIA-Vis Images.

A selfie-ready skin.

Nowadays, taking selfies using your mobile phone and posting them on social media is a very common practice, especially among young people. Therefore, it is both **interesting and innovative** to include in our *in vivo* study that **volunteers took a selfie every day**, before applying the treatment, and that they also reviewed the evolution of the 42 resulting pictures.

As a result, at day 42, Trikenol™ Plus **reduced the amount of acne imperfections by 35% and the area affected by these imperfections by 19%**, versus placebo.





D0  D42

Selfies taken by the volunteers.

CONCLUSIONS



Trikenol™ Plus reduces acne to allow you to take the perfect selfie, every time.

TRIKENOL™ PLUS: Your best picture to share with friends.

COSMETIC APPLICATIONS

- ✓ Young and mature skin
- ✓ Anti-acne treatments
- ✓ Sensitive skin
- ✓ Seboregulating products
- ✓ Microbiota protection

RECOMMENDED DOSAGE

The recommended dosage is 0.8 %.

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