BASF
We create chemistry

Care Creations™

Bix'Activ®

The cutaneous diet for a pure and mattified skin

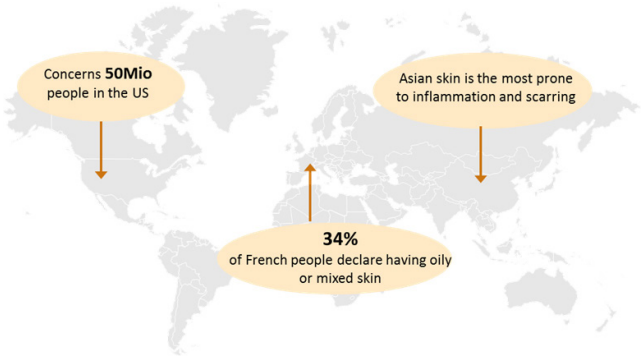
by Beauty Creations
another Care Creations™ product group Inspired by Life

TABLE OF CONTENTS

SUMMARY FILE

Bix'Activ®	**BC10050**
Origin - Description	Standardized natural extract of *Bixa orellana* seed
Regulatory data	
INCI	Maltodextrin (and) Bixa Orellana Seed Extract
China	Each component is listed in "Inventory of Existing Cosmetic Ingredient in China" (IECIC 2015)
CAS#	9050-36-6; 89957-43-7
EINECS#	232-940-4; 289-561-2
Appearance	Beige to yellowish fine powder
Preservative	None
Natural labels	Raw material conform to COSMOS standard of natural and Organic Cosmetics
Naturalness content (ISO 16128)	100% from natural origin
Cosmetic use	
Properties	Decrease sebaceous gland activity Decrease pore size Decrease skin imperfections Maintain skin hydration Mattify the skin
Applications	Mattifying cream Anti-oily skin treatment Night sebo-control treatment Suitable for Multi-Ethnic solutions
Formulation data	
Concentration of use	0.25%
Solubility	Soluble in water
Incorporation method	Dissolved at 20% w/w in water at room temperature and then incorporated during the final process below 30°C, or at room temperature for cold processing
Optimal pH	3-8
Patent family	Application filed

OILY SKIN, A GLOBAL CONCERN



Bickers *et al.*, 2006, Richard *et al.*, 2017, Goh *et al.*, 2016.

Oily skin (seborrhea) is a common and worldwide cosmetic problem affecting up to 80% of young people, in their teens and early 20's (Nouveau-Richard et al., 2007). However, it's not unusual to have oily skin into one's 30's and beyond. 35% of the global population suffers from oily skin. Even 58% of women worldwide between 25 and 34 are concerned.

Seborrhea, mostly often associated with skin imperfections concerns both genders and all ethnicities (Caucasian, Asian and Black people), even if it can vary among these groups. For example, 25% of Chinese women are concerned (Nouveau-Richard et al. 2007). Additionally, environmental factors, such as pollution and hot humid climates, stress or daily habits such as diet also influence oily skin.

Three clinical signs are linked to oily skin, shininess, enlarged pores and imperfections. These signs of oily skin, associated with a perception of "dirty" skin, negatively affect the self-image of people and have detrimental psychosocial effects. People with oily skin often complain of an unclean feeling (Sakuma and Maibach, 2012).

THE ORIGIN OF OILY SKIN IS LINKED TO SEBACEOUS GLANDS

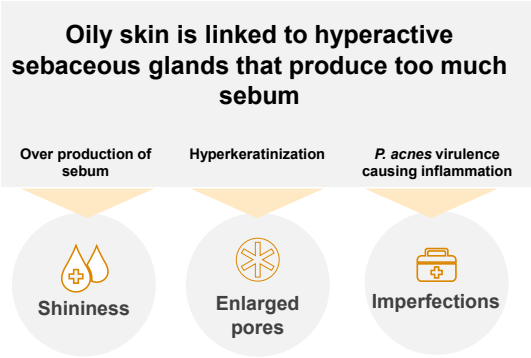


Figure 1 - Three main characteristics of oily skin.

Seborrhea occurs when oversized sebaceous glands produce excessive amount of sebum due to a hyperactivity of its specialized cells, the sebocytes. This leads to a shiny and greasy skin which is associated with enlarged visible pores, and a breeding ground for imperfections such as pimples, blemishes or even acne. Furthermore, oily skin is characterized by a thick complexion correlated to epidermis hyperkeratinization (Figure 1).

Sebaceous glands: the Center of Sebum Secretion, a Complex Mixture of Lipids

Location of sebaceous glands

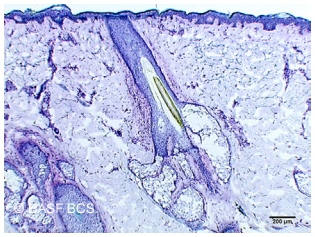


Figure 2 - Sebaceous gland in normal human skin biopsy. Hematoxylin-eosin staining.

Sebaceous glands are mostly located in skin all over the human body, even in hair-covered and hairless areas. They are more numerous on the scalp and face reaching up to 400-900 glands/cm^2 whereas they are less than 50/cm^2 on the forearm (Smith TM *et al.*, 2008). The oily appearance commonly found on the 'T' zone (forehead, nose and chin) reflects the dominance of these sebaceous glands. In the skin, they are located in the reticular dermis where they are usually found in association with hair follicles, forming the pilosebaceous unit, both are then dependent from each other (Figure 2).

Function of sebaceous glands: sebum production

The main function of sebaceous glands is the secretion of sebum through a holocrine breakdown of mature sebocytes (Smith TM *et al.*, 2008). The complete renewal of sebocytes in sebaceous glands lasts around 4 weeks (Epstein EH and Epstein WL, 1966). The fully developed adult sebaceous gland contains sebocytes at different stages of differentiation. In fact, peripheral sebocytes proliferate (mitotic activity) and then differentiate towards the center of the gland (Smith TM *et al.*, 2008). During this process of maturation, they lose their mitotic activity, increase their size and accumulate lipid droplets (Smith TM *et al.*, 2008). Once sebocytes are mature, the membranes disrupt, sebocytes disintegrate and die, and release their sebum via holocrine secretion into the duct of the hair follicle thence to the skin surface. The quantity of lipids delivered in a given time per unit area is proportionate to the total glandular volume (size and number of glands). This continuous maturation activity is under the control of hormones, cytokines, signaling molecules and mediators of the lipid metabolism, and are affected by various factors from environment and diet.

Sebaceous gland characterization

Normal human proliferating sebocytes, express Ki67, as usual mitotic cells. Once they progress in the differentiation stage, they express markers of sebaceous lineage, cytokeratin 7 (K7) and, epidermal membrane antigen (EMA), also called Mucin-1 (Muc-1). K7 characterizes the undifferentiated cells present at the periphery of the gland and Muc-1 characterizes the terminal sebocyte differentiation present at the center of the gland (Figure 3 and Figure 4). K7 is expressed in the cell cytoplasm. Muc-1 is expressed at the sebocyte membrane, and helps the migration of the sebocytes in the center of the gland (Jung *et al.*, 2017). At the center, the cell volume increases and lipids are synthesized to produce sebum. They can subsequently undergo apoptosis.

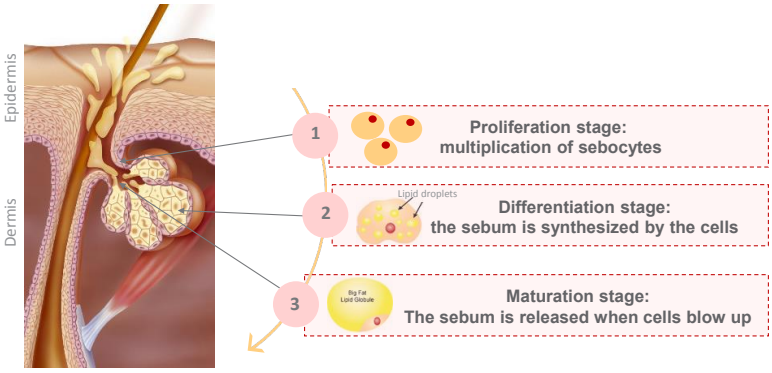


Figure 3 - The different differentiation stages of sebocytes in the skin.

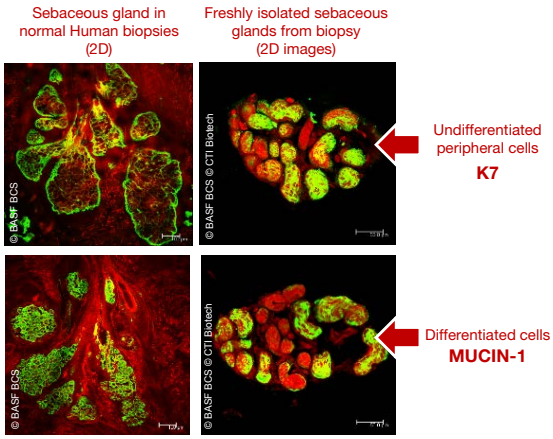


Figure 4 - Immunofluorescence staining (green) of the markers K7 and Muc-1 in sebaceous glands in normal human biopsies and in freshly isolated sebaceous glands from normal human biopsies. Counter coloration in red.

Sebaceous gland regulation – different signaling pathways

Androgen pathway

The sebaceous gland is an androgen target organ, stimulated to produce sebum at puberty and beyond androgens (Zouboulis *et al.*, 1998). Akamatsu *et al.*, (1992) demonstrated that the androgen factors, testosterone and 5-alpha-dihydrotestosterone (5α-DHT) stimulated the proliferation of facial cultured human sebocytes in a significant dose-dependent manner. Androgens are more effective in increasing the proliferation of facial than non- facial sebocytes (Zouboulis *et al.*, 1998, Akamatsu *et al.*, 1992).

Insulin and Insulin growth factor pathways

Besides androgen hormones, other factors modulate sebocytes activity, leading to a complex regulation of the sebaceous gland. Indeed, insulin and insulin growth factor-1 (IGF-1) levels also peak during late puberty and gradually decline until the third decade (Zouboulis *et al.*, 1998, Smith *et al.*, 2007). Insulin and IGF-1 stimulate sebocyte proliferation and sebaceous gland sebogenesis *in vitro* by regulating numerous genes involved in lipid biosynthesis (Smith KR *et al.*, 2008).

IGF-1 also mediates the induction of androgen production and stimulation of peripheral androgen metabolism. Thus, IGF-1 has direct influence on the intracrine androgen regulation of the skin and potentiates androgen signaling by the induction of 5α-reductase activity and activation of the androgen receptor (Smith *et al.*, 2007).

The expression of IGF-1 receptors on the sebocytes determines whether they respond to IGF-1. Interestingly, the keratinocytes also express the IGF-1 receptor, and IGF-1 is essential for the development of a normal epidermis. IGF-1 in the skin is produced by dermal fibroblasts and epidermal melanocytes (Tavakkol *et al.*, 1992), and dermally derived IGF-1 has been shown to stimulate keratinocytes *in vitro* (Barreca *et al.*, 1992). Keratinocytes do not synthesize IGF-I but are highly responsive to IGF-1 through their IGF-1 receptors, which are most abundant in the basal layer (Krane *et al.*, 1992).

Interestingly, as with most tissues studied so far, IGF-1 action in the epidermis occurs in the presence of IGF-I binding proteins (IGFBPs). IGFBPs serve as carrier proteins for IGFs and thus modulate their bioactivity. In the absence of added growth factors, IGFBP-3 is the major IGFBP produced by cultured human keratinocytes. IGFBP-3 is produced by basal keratinocytes *in vivo*, and it is the major IGFBP expressed in the epidermis. IGFBP-3 binds to IGF-1, and prevents IGF-1 binding to its receptor, preventing keratinocyte hyperproliferation and hyperdifferentiation.

Sebum functions

Sebum lubricates the skin and scalp and traps moisture. Indeed, sebum helps keep the skin flexible and prevents water loss from the body. The major function of lipids in skin epidermis is to form a permeability barrier between the external environment and the internal milieu; therefore, alteration in the metabolism of these lipids can lead to skin dysfunction (Shi *et al.*, 2015). *Stratum corneum* hydration declines in areas of human skin having decreased sebaceous glands, and glycerols derived from triglycerides in sebaceous glands play an important role in skin hydration (Choi *et al.*, 2005).

Sebum lipid composition

Sebum is a unique complex mixture of lipids with a high proportion of triglycerides (30-50%), free fatty acids (FFA) (15-30%), wax esters (26-30%) and squalene (12-20%), 3.0% cholesterol esters and 1.5% cholesterol (Picardo *et al.*, 2009). Among these, squalene and wax esters are unique to human sebum and not found anywhere else in the body nor among the epidermal surface lipids which composition is different (comprised of 50% ceramides, 25% cholesterol, 15% of free fatty acids (Feingold, 2007) as well as smaller amounts of cholesterol esters and cholesterol sulfate). Notice that squalene is considered to be a marker for sebocyte differentiation and thus for sebogenesis.

Sebaceous glands and microbiome

Epidermis, sebaceous glands and hair follicles are likely associated with their own unique microbiota. Sebaceous glands support the growth of facultative anaerobes such as *Propionibacterium acnes* (*P. acnes*), a common skin commensal bacterium (Liu *et al.*, 2015). The lipases of *P. acnes* degrade skin lipids of sebum. They especially hydrolyze the triglycerides present in sebum, releasing FFA onto the skin. The bacterium can then adhere to these FFA, and this perhaps aids in the colonization of the sebaceous gland by bacteria. These free fatty acids also contribute to the acidic pH (~5) of the skin surface which participates to skin irritations.

Sebum level changes throughout life

Differences in sebum secretion at various stages of life have been associated with concomitant changes in endogenous androgen production (including testosterone) and also in insulin and IGF-1 production. Sebaceous glands are well developed in neonates, but their size decreases dramatically a few weeks after birth, start to rise again and reaches its maximum in young adults. While the number of sebaceous glands remains the same throughout life, sebum secretion rates are highest in 15-35 year olds and decline continuously throughout the adult age range (Jacobsen *et al.*, 1985). At any age range, the mean sebum values in men exceed those of women. Aging skin displays morphological changes and alterations in sebaceous glands. These physiologic changes showing reduced sebaceous gland activity correlate with the period in which the aging adult develops xerotic dermatitis.

Sebum level depends on ethnicity

Whatever the ethnic group, the sebaceous excretion rate is lower on the cheek than on the forehead. Sebum level is lower in Chinese than in African- American, Mexican and Caucasian people on the cheek and forehead (Sakuma TH and Maibach HI, 2012). Moreover, African American women secreted larger amounts of sebum than Caucasian women. Hillebrand *et al.* (2001) reported that African-Americans showed significantly more sebum excretion than East Asians. The level of the wax esters was significantly higher in African American women (Pappas *et al.*, 2013).

Factors triggering oily skin and associated issues

Oily skin is characterized by excessive sebum production with abnormal lipid ingredients which contributes to the formation of blemishes, in addition to the hyperkeratinization of the epidermis. Besides surface oiliness, excess sebum blocks pores, provides nourishment to *P. acnes* that lives upon the skin and contributes to oily skin and serious acne flare ups.

Sebaceous glands and sebocytes are affected by hormones, growth factors and lipid mediators

An excess of androgen hormones and growth factors participate to oily skin development, particularly during puberty where the levels of these hormones and growth factors are high (Figure 5).

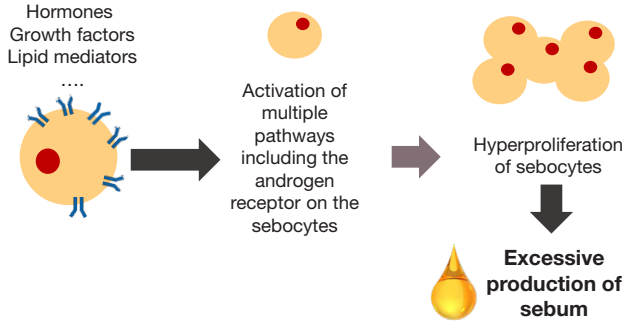


Figure 5 - Hormones, growth factors and other signaling factors induce sebocyte hyperproliferation and sebum overproduction.

IGF-1, is also considered to be a growth hormone of puberty, inducing the synthesis of androgens and enhancing 5α-reductase activity in the skin (Melnik and Schmitz, 2009). Indeed, excess IGF-1 is associated with an increased sebum production (Smith KR *et al.*, 2008).

IGF-1 serum levels also correlate directly with the amount of facial sebum in both men and women (Vora *et al.*, 2008).

Impact of our diet and environment on excessive production of sebum

Melnik (2012) showed that high milk- / dairy protein intake and high glycemic load lead to IGF-1 hypersecretion and to the development of oily skin (Figure 6). Indeed, there is a link between the Westernization of dietary patterns and the development of oily skin and acne (Melnik, 2012).

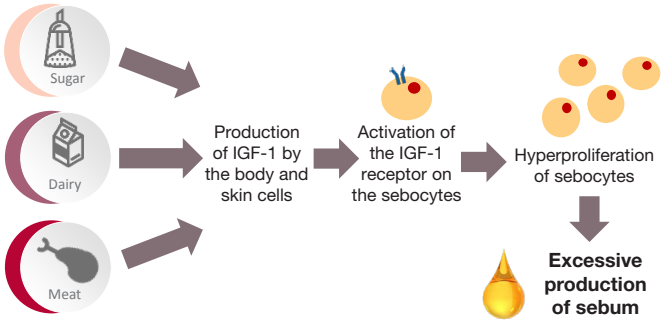


Figure 6 - Sebaceous glands are affected by our diet through IGF-1 signaling pathway.

A study describes also a correlation between IGF-1 serum levels and the severity of acne in women (Cappel *et al.*, 2005).

Upon oxidative challenge (e.g. UV radiation, pollution), squalene is readily oxidized to squalene peroxide, which is comedogenic. Squalene is the first human skin surface lipid oxidized e.g. by sun. In vitro data showed that squalene peroxide beyond the induction of HaCaT keratinocyte proliferation also leads to the upregulation and release of inflammatory mediators, which indicate a pro-inflammatory activity of by-products of squalene oxidation (Ottaviani *et al.*, 2006).

In recent years, an increasing number of studies indicate a link between skin problems (including oily skin and acne) and exposure to airborne pollutants, such as particulate matter (PM), volatile organic compounds, ozone (O_3), nitrogen dioxide (NO_2) and sulfur dioxide (SO2) (Xu *et al.*, 2011).

Impact of our diet and environment on hyperkeratinization around pores

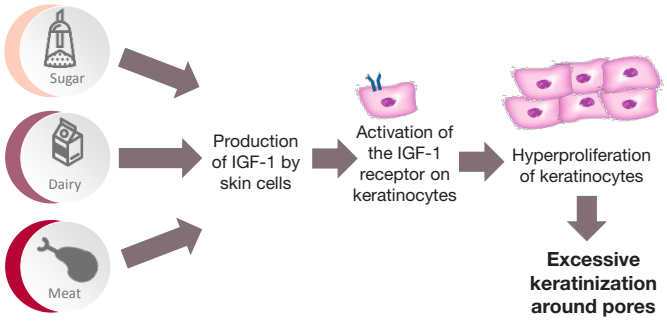


Figure 7 - Excessive keratinization around pores is affected by our diet.

In the skin, IGF-1 induces keratinocyte proliferation *in vitro* (Barreca *et al.*, 1992) and *in vivo* (Sadagurski *et al.*, 2006). Moreover, IGF-1 is a strong stimulator of skin hyperkeratinization (Isard *et al.*, 2011) that can be linked to conspicuous pores (Figure 7).

The central role of microbiota in oily skin related issues

Propionibacterium acnes (*P. acnes*) is a commensal bacterium colonizing human sebaceous glands (Nouveau-Richard *et al.*, 2007; Sakuma and Maibach, 2012).

Lipase is a key virulence factor secreted by *P. acnes*. This enzyme catalyzes the release of free fatty acids (FFA) from sebum triglycerides (Picardo *et al.*, 2009). Released FFA such as palmitic acid can induce inflammation and keratinocyte proliferation, while oleic acid stimulates adhesion and keratinocyte proliferation, thereby promoting comedogenesis. A study showed that the level of lipase secreted by *P. acnes* is positively correlated with the severity of acne lesions and the virulence of *P. acnes* biotypes (Higaki *et al.*, 2000) Thus, the research of lipase inhibitors constitutes a relevant way to decrease the inflammatory process and comedogenesis in sebaceous glands.

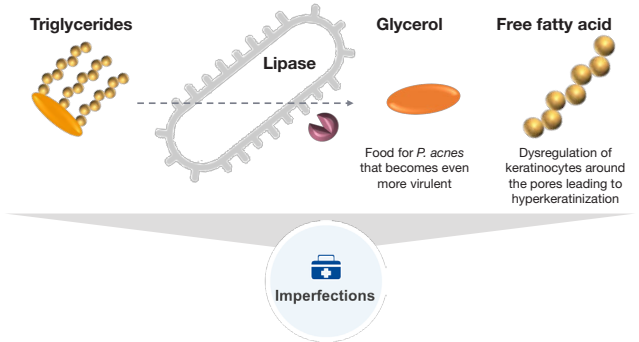


Figure 8 - The central role of *P. acnes* in oily skin. Secreted lipase triggers skin imperfections.

Sebaceous triglycerides and released fatty acids hydrolyzed by *P. acnes* can enter the follicular wall and be incorporated into the metabolism of the surrounding epidermis. Unsaturated fatty acids may increase the concentration of calcium in keratinocytes, leading to abnormal follicular keratinization (Jarousse *et al.*, 2007). The formed FFA are irritants and inflammatory and participate in skin imperfections (Figure 8).

Interestingly, *P. acnes* induces IGF-1R expression and IGF-1 secretion by keratinocytes (Isard *et al.*, 2011), and therefore may take part in hyperkeratinization in oily skin through the IGF-1 signaling pathway.

Challenge & Strategy

Oily skin is a global concern which is exacerbated by hormonal changes but also by various environmental and lifestyle factors such as a diet rich in dairy or sugar foods, alcohol, stress, pollution, weather...

To answer to this concern, our challenge was to develop a solution targeting the main pathways triggering oily skin and its clinical signs: shininess, enlarged pores and imperfections (Figure 9).

Bix'Activ corrects oily skin by acting on the sebaceous gland to reduce sebum overproduction, on keratinocytes to reduce hyperkeratinization and the size of pores, and on *P. acnes* virulence.

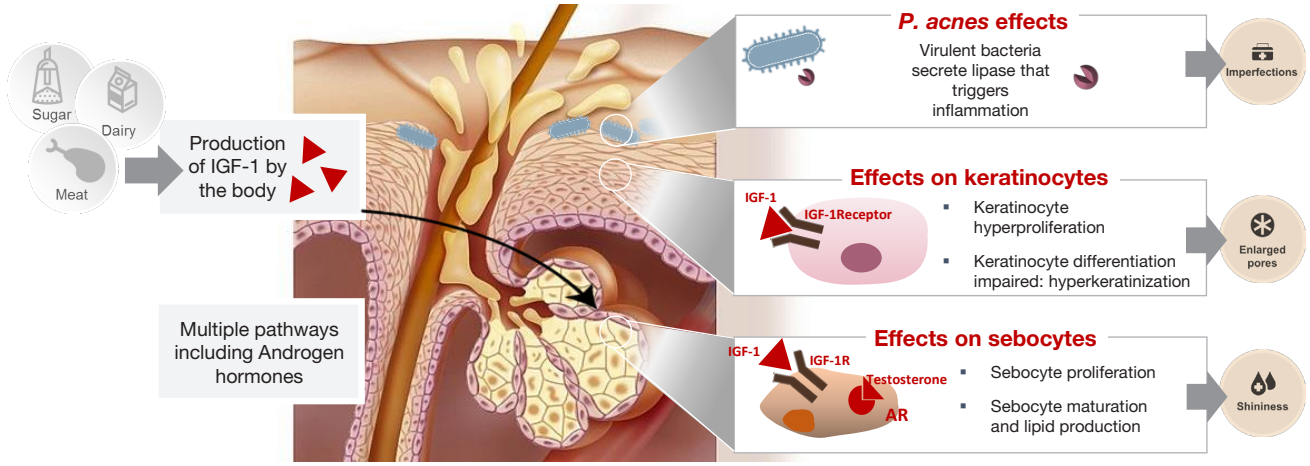


Figure 9 - Main pathways that triggers oily skin associated issues.

BIX'ACTIV, A NATURAL INGREDIENT FOR A MATTE AND REFINED SKIN

Description of the plant

Family: Bixaceae

Species: *Bixa orellana*

Common name: Roucou, Achiote, Red lip tree

Description: *Bixa orellana* is an evergreen shrub up to 8 m high, it bears clusters of white to pink flowers. The not edible fruits are in clusters of red-brown seed pods covered in soft spines. Each pod contains many seeds covered with a thin waxy red aril rich in carotenoids. At maturity, the pod dries, hardens, and splits open, exposing the seeds.

Origin: Burkina Faso

Plant part used: Seeds

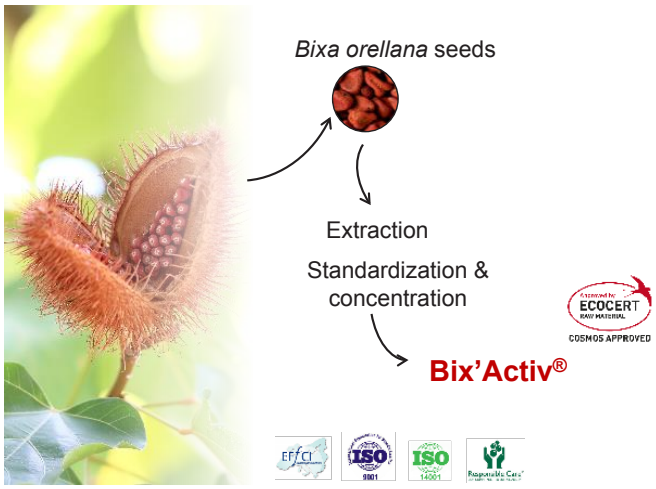
Distribution: The plant native to tropical America but grows in other regions of South and Central America and Africa.

Uses: *Bixa orellana* has an extensive agro-industrial value because its seeds have a high carotenoid content, mainly composed of bixin. The plant has been used since ancient Mayan civilization times as a culinary colorant and spice, body paint and for healing purposes such as bladder conditions, fevers, dysentery, burns, inflammation, heartburn and various skin problems.

It is currently used as a natural pigment in food, in pharmaceutical, and cosmetic industries, and it is commercially known as annatto. The most documented properties of this natural pigment are anti-oxidative; but its anti-cancer, hypoglycemic, antibacterial, digestive and anti-inflammatory properties are also being studied.

Sourcing: The fruits are collected from November to December on domesticated trees in the west part of Burkina Faso. Then the fruits are manually opened to release the seeds. The seeds are then sun dried.

Processing and Composition



Bix'Activ is obtained through a nature-friendly extraction process using water as sole solvent. Following a first step of aqueous extraction of the grinded seeds, the unextracted insoluble matter is removed and the product standardized and concentrated to obtain Bix'Activ as a preservative-free powder with maltodextrin (30-50% Bixa Orellana Seed Extract; 50-70% Maltodextrin. This process is conducted also to get rid of dyes largely present is the seeds.

As shown in figure 10 which represents the typical phytochemical composition, the plant extract matter contains mostly carbohydrates, with a characteristic presence of uronic acids (sugar acids with a carboxylic functional group). A final content of uronic acids ≥ 1.3% is used to characterize Bix'Activ after product standardization with maltodextrin.

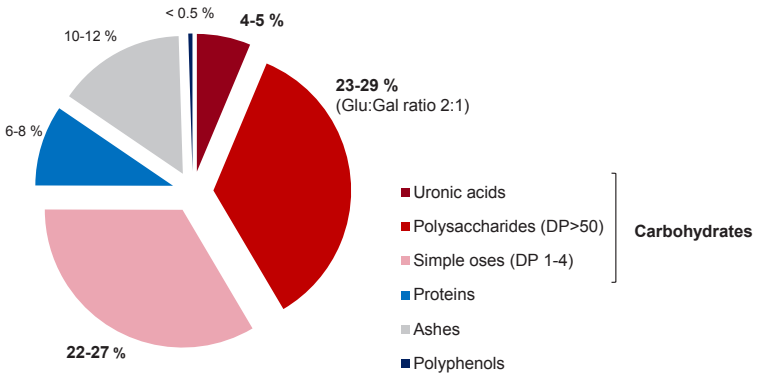


Figure 10 - Typical phytochemistry composition of the plant extract matter (based on three representative batches) in dry weight percentages.

Further analysis of Bix'Activ's carbohydrates have shown that they are composed mostly of polysaccharides of high degree of polymerization (DP>50). The saccharide components of these polysaccharides are predominantly Glucose (Glu) and Galactose (Gal) in a 2:1 w:w ratio, with Mannose as a minor component.

Safety / tolerability of the product

Bix'Activ was tested to ensure its safety in the recommended conditions of use. Bix'Activ does not irritate the eyes or skin and no indication of skin sensitization was observed.

Description

Bix'Activ is a natural standardized preservative-free extract of *Bixa orellana* seeds.

INCI name: Maltodextrin (and) Bixa Orellana Seed Extract

Naturalness content (according to ISO 16128): 100% from natural origin

Dose of use: 0.25%

China IECIC listed

Approved by ECOCERT RAW MATERIAL COSMOS APPROVED

Example: Illustration of a commercial sample of Bix'Activ® with an emulsion and hydrogel containing 0.25%.

DEMONSTRATED EFFICACY

Bix'Activ has been proven to reduce sebum production, shininess, pore size and imperfections while maintaining skin hydration *in vivo*.

Proven *in vitro* performance

Bix'Activ has proved its efficacy *in vitro* to reduce lipid synthesis in sebocytes (in 2D and 3D models). It is efficient regardless of ethnic origin of the cells and through 2 signaling pathways inducing sebum overproduction:

- male human serum enriched in testosterone,
- IGF-1 pathway related to dietary habits.

Bix'Activ is efficient in reducing keratinocyte hyperkeratinization *in vitro* by targeting a specific secreted protein of the IGF-1 signaling pathway, IGFBP-3.

Bix'Activ inhibits the lipase activity of *P. acnes in vitro*, which reflects the virulence of the bacterium involved in the hyperkeratinisation process.

Proven *in vivo* performance

In vivo, Bix'Activ was evaluated for its sebum regulation and mattification properties while maintaining the hydration level on both Asian and Black skin. In addition, Bix'Activ reduces the size of pores and imperfections in Asian skin.

EFFICACY

Reduction of induced-lipid overproduction in normal human sebocytes regardless of ethnicity

in vitro

OBJECTIVE

First, we built a sebocyte model in 2D culture where the proliferative rate of sebocytes and the quantity of lipid droplets were stimulated, in order to mimic an overproduction of sebum *in vitro* as observed in oily skin. Two signalling pathways were explored: Human Serum (HS) which is enriched in testosterone (androgen factor, between 10 and 30 nmol/L) and Insulin Growth Factor (IGF-1) which is a survival pathway known to be partly stimulated by a hyperglycemic and milk diet (Smith *et al.*, 2007).
Second, we investigated the effects of Bix'Activ on the proliferative rate of sebocytes and on the quantity of lipid droplets in normal human primary sebocytes in culture stimulated with either HS or IGF-1. Different ethnicities of sebocytes were studied.

RESULTS & DISCUSSION

Validated model for lipid overproduction

Cell number (Figure 11A) and total lipids (Figure 11B) were significantly induced ($p<0.001$) by 1.8 and 2.6-fold in HS-induced sebocytes and by 1.6 and 4-fold in IGF-1 induced sebocytes respectively.

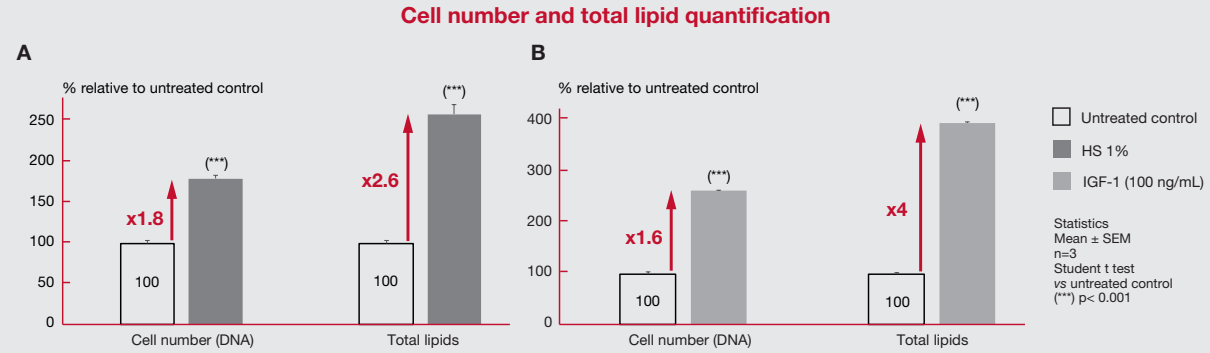


Figure 11 - Cell number and total lipid quantification in normal human primary sebocytes. Comparison of untreated cells (100%) vs HS- (A) or IGF-1- treated sebocytes (B).

Effects of Bix'Activ on normal human sebocytes from different ethnic origins regulated either by HS or by IGF-1

When Asian sebocytes were treated with HS 1%, increased lipid accumulation in the cytoplasm was detected by microscopy after staining with Nile red (Green fluorescence, Figure 12). Bix'Activ at 0.02% decreased the number of lipid droplets in HS- induced sebocytes. These visualized immunostainings were confirmed with a quantification (Figure 13). Indeed, Bix'Activ decreased significantly ($p<0.001$) sebocyte proliferation and lipid production by 16% and 17% respectively in Asian HS- induced sebocytes.

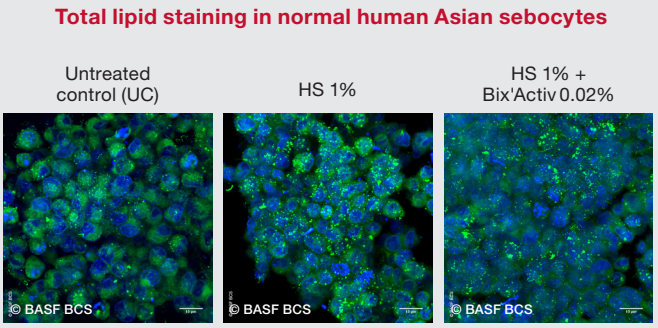


Figure 12 - Immunostaining of nuclei (in blue) and lipid droplets (in green) in normal human Asian sebocytes treated with Human serum (HS) ± Bix'Activ®.

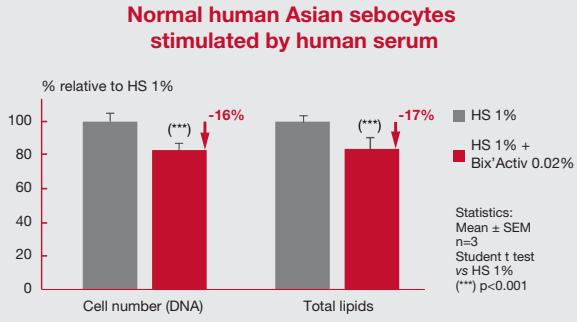


Figure 13 - Cell number and total lipid quantification in normal human Asian sebocytes stimulated by Human serum (HS) ± 0.02% of Bix'Activ.

in vitro

Figure 14 shows the results obtained when the sebocytes are stimulated by IGF-1. We confirmed that Bix'Activ was also able to inhibit sebocyte proliferation and lipid production in IGF-1-stimulated Asian sebocytes. At 0.02%, Bix'Activ significantly inhibited sebocyte proliferation by 15% ($p<0.001$) and lipid synthesis by 12% ($p<0.05$).

On sebocytes from an Asian donor, Bix'Activ significantly inhibited lipid production in both HS and IGF-1- activated cells.

Normal human Asian sebocytes stimulated by IGF-1

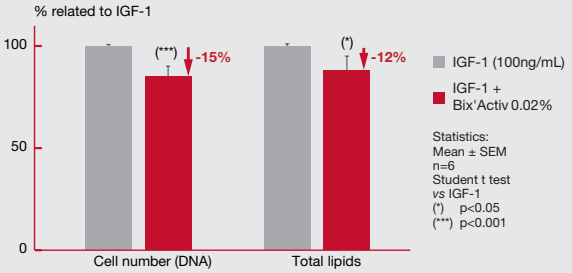


Figure 14 - Cell number and total lipid quantification in normal human Asian sebocytes induced by IGF-1 ± 0.02% of Bix'Activ.

Figure 15 shows the effects of Bix'Activ on HS-stimulated sebocytes originated from a Black American female donor. We calculated the number of lipid droplets depending on the size range of the droplets. The culture gave rise to larger number of small droplets (< 10 μm^3) compared to the number of the big ones (> 10 μm^3) regardless of culture treatment. Especially, we showed that Bix'Activ significantly inhibited ($p<0.001$) the synthesis of lipid droplets whose size is comprised between 0 and 30 μm^3. The decrease was also observed for the biggest lipid droplets but the small quantity of these droplets couldn't give statistically significant data. In total, the number of lipid droplets decreased significantly by 28% ($p<0.001$).

These results suggest that Bix'Activ has an effect from lipid droplet neo-genesis to their maturation.

Total lipid droplet quantification in normal human Black American sebocytes stimulated by human serum

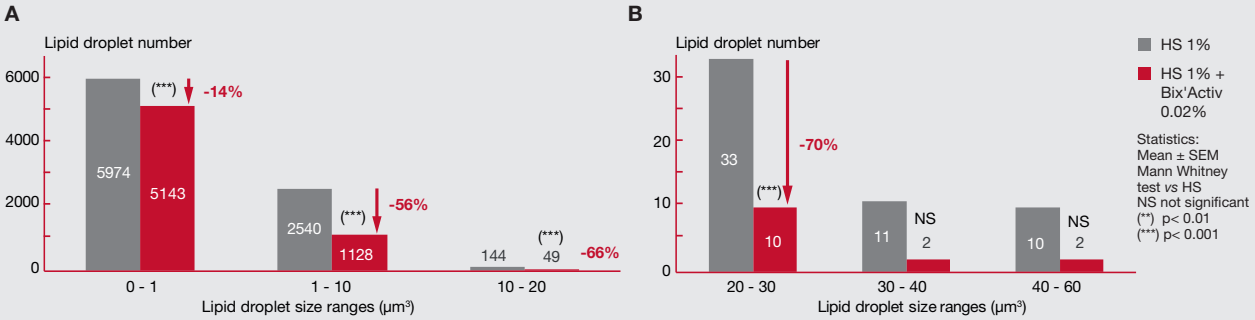


Figure 15 - Lipid droplet quantification in normal human Black American sebocytes induced by HS ± 0.02% of Bix'Activ. **A** - Small Lipid droplets are separated by size ranges between 0 and 20 μm^3. **B** - Big Lipid droplets are separated by size ranges between 20 and 60 μm^3.

In Caucasian sebocytes, Bix'Activ at 0.02% inhibited significantly sebocyte proliferation and lipid synthesis ($p<0.001$ and $p<0.05$ respectively) by 11% in IGF-1-stimulated sebocytes (Figure 16).

Normal human Caucasian sebocytes stimulated by IGF-1

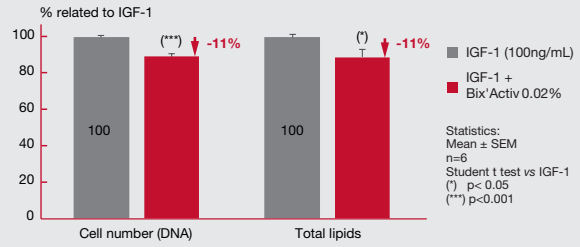


Figure 16 - Cell number and total lipid quantification in normal human Caucasian sebocytes induced by IGF-1 ± 0.02% of Bix'Activ.

CONCLUSION

Bix'Activ regulates lipid production on normal human primary sebocytes through the inhibition of sebocyte proliferation. The inhibition is observed regardless of sebocyte ethnicity in both HS and IGF-1 pathways.

Through these both pathways reported to be stimulated by hormonal changes and sugar or dairy-rich diets, Bix'Activ may contribute to reduce sebum overproduction in oily skin.

MATERIALS&METHODS

Cell culture

Normal human primary sebocytes (Asian woman, 28 years old, face, or Black American, face, 28 years old, or Caucasian woman, face, 25 years old) were seeded at 2500 cells/cm² within complete medium (DMEM/HAM F12) with FCS at 10% and incubated for 5 days at 37°C, CO_2, 5%. Growth medium was replaced by a standard medium (DMEM) containing either 1% human serum (HS) or IGF-1 at 100 ng/ml for an additional 5 days. When the cells were treated with Bix'Activ, it was incorporated at the same time HS or IGF-1.

DNA staining and quantification

The cell number was determined by DNA staining with Hoechst reagent and the fluorescence was recorded at 465 nm (excitation at 356 nm).

Total lipid staining and quantification

The quantity of cellular lipids was determined with Nile red reagent. We used an excitation light at 810 nm (Femtosecond laser) and emission fluorescence between 590-654 nm. The fluorescence was recorded at 625 nm (excitation at 520 nm).

For lipid counting analysis, for each condition and each stack on the Nile Red channel, we made a 3D segmentation to isolate the lipid droplets. Then we generated some statistics to describe it (size, volume, distribution). All measures were done with AMIRA software (Thermo Fisher).

Statistics

The results are normalized to the control medium alone and normalized to the basal medium with either HS 1% or IGF-1 at 100 ng/mL. The results are expressed as mean (%) ± standard deviation of the mean (SEM). The statistical analysis was done after normal distribution comparison of the values following SigmaPlot software recommendations (Systat Software Inc. USA). The threshold of significance was set at 5%.

EFFICACY

Reduction of induced-lipid overproduction on a 3D mature sebaceous gland model

ex vivo

OBJECTIVE

Why work with 3D sebaceous gland model?
Cultured human sebocytes have been shown to preserve important sebocytic characteristics, although they undergo an incomplete terminal differentiation *in vitro* (Xia *et al*., 2009).
We developed for the first time a 3D sebaceous gland model ("Sandwich" method developed in collaboration with CTIBiotech) which resulted in the production of physiological sebum, able to generate squalene (between 4 and 11 µg/mg of tissue depending on donor phototypes II, IV and V, data not shown). This is the first 3D model with sebaceous glands in culture, close to skin physiology, used to study the effect of an active ingredient. A real 3D visualization of the sebaceous glands was performed to observe the maturation marker, MUC-1 in the glands.

RESULTS & DISCUSSION

Figure 17 showed that Bix'Activ affected MUC-1 synthesis, the maturation marker expressed in the center in the sebaceous gland (yellow staining). Bix'Activ decreases MUC-1 synthesis, therefore, may decrease the sebocyte maturation.

Muc-1 immunostaining

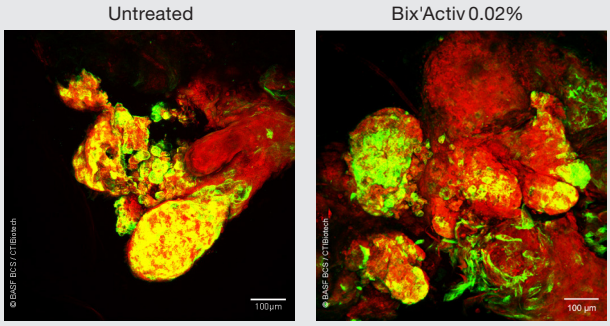


Figure 17 - Sebaceous glands cultured in a "Sandwich" method for 6 days. Mucin-1 immunofluorescence (green) staining. Counter coloration in red.

In figure 18, with Nile red staining, we showed that Bix'Activ decreased the density of lipids in the sebaceous gland. The sebaceous gland appeared depleted of its sebum.

Lipid droplets and nuclei staining

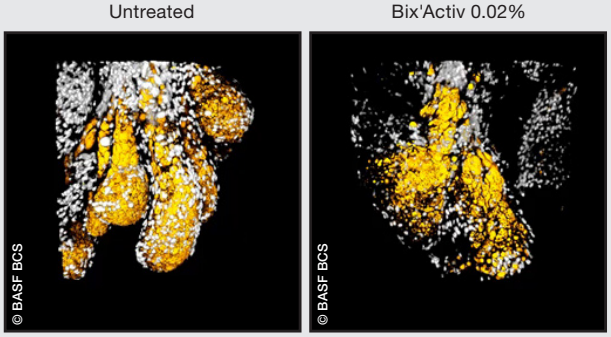


Figure 18 - Sebaceous glands cultured in a "Sandwich" method for 6 days. Lipid droplets stained with Nile Red (yellow) and nuclei with Dapi (white).

CONCLUSION

We confirmed *ex vivo* on sebaceous glands the physiological action of Bix'Activ. It decreases the maturation of the sebocytes at the centre of the gland, and the production of the sebum.

MATERIALS&METHODS

3D Analysis of the sebaceous glands in culture

3D sebaceous gland in culture, "3D Sandwich model"

Sebaceous glands (woman, face, 58 days, phototype II) were microdissected from a skin sample in aseptic conditions. Epidermal and dermal tissue around sebaceous glands were progressively removed with scalpels under a stereomicroscope in aseptic conditions. Sebaceous glands were then transferred into 6-well plates coated with fibronectin. Sebaceous glands were cultivated between a fibronectin coated plate bottom and a fibronectin-coated coverglass with a 1mL culture medium ("Sandwich" ex vivo culture). 3 glands were grown in each well. The medium was changed every 3-4 days. The culture was continued for 6 days.

Mucin-1 (MUC-1)

A histological study was carried out on isolated sebaceous glands treated or not with Bix'Activ for 5 days. After extraction and treatment of the sebaceous glands, the samples were fixed in paraformaldehyde at 4%. After several washes in PBS, the samples were placed in a serum solution. The primary antibody anti-MUC-1 was incubated overnight at room temperature. After several washes with PBS, the secondary antibody coupled with Alexa 488 was also applied overnight at room temperature and in dark conditions. The Evans blue (counter coloration) was applied after several washes for 5 min at room temperature. after the last wash, the samples were placed on a coverslip with Fluoprep for observations using confocal microscope (TCS-SPE, Leica) (cf Figure 17).

Lipids and nuclei stainings with 3D analysis

After Nile Red (lipids) and Dapi application (nuclei), imaging was done with a confocal microscope (LSM780, Zeiss) using the excitation laser at 405nm and 561nm and an objective 63X /1.40 oil. We acquired two stacks of images per area for both channels. Each stack of images was treated to obtain a z-projection on maximal intensity to obtain a 3D visualization (Figure 18).

We made 3D reconstruction of the sebaceous glands in each condition (AMIRA software) and a video was realized (Adobe PremierePro) to observe the sebaceous glands on all sides. Pictures presented here are taken from the video

EFFICACY

Induction of IGFBP-3 secretion in normal human keratinocytes to reduce hyperkeratinization

in vitro

OBJECTIVE

To address hyperkeratinization, we looked at the IGF-1 pathway activated by dairy and sugar-rich diet.
Because IGFBP-3 inhibits IGF-1-induced keratinocyte hyperproliferation and hyperkeratinization in the epidermis, we tested whether Bix'Activ was able to induce IGFBP-3 secretion in order to counteract the effect of IGF-1 in the skin.

RESULTS & DISCUSSION

Figure 19 shows that Bix'Activ significantly activated ($p<0.05$) the secretion of IGFBP-3 by 1.7 fold in normal human primary keratinocytes.

CONCLUSION

Bix'Activ, by its positive effect *in vitro* on IGFBP-3, may be able to block IGF-1 signaling, and thus to decrease the proliferation and hyperkeratinisation of keratinocytes which is observed in oily skin, and involved in the appearance of enlarged pores.

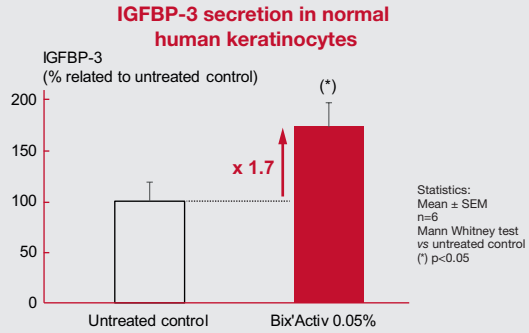


Figure 19 - Effect of Bix'Activ on IGFBP-3 protein in normal human keratinocytes.

MATERIALS&METHODS

Cell culture

Human normal keratinocytes, from an African American donor of 19 years old, were cultured in KSFM medium (Gibco). Cells were grown and amplified at 37°C and 5% CO_2. Keratinocytes were seeded in 24 well plates with KSFM medium (Gibco) for treatments. Cells were treated in the proliferation phase for 24h, with or without Bix'Activ at 0.05%. Then the supernatants were harvested for IGFBP-3 assay by ELISA (LSBio Human IGFBP-3 ELISA Kit ref LS-F24538), Optical density was read at 450 nm. The supernatant from untreated cells was used as a control. The percentages were expressed in % relative to untreated control.

Statistics

The results are normalized to the control medium alone. 6 independent experiments were carried out. The results are expressed as the mean (%) ± standard deviation of the mean (SEM). The statistical analysis was done after normal distribution comparison of the values following SigmaPlot software recommendations (Systat Software Inc. USA). The threshold of significance was set to 5%.

EFFICACY

Inhibition of *Propionibacterium acnes* virulence

in vitro

OBJECTIVE

Propionibacterium acnes (*P. acnes*) is a commensal bacterium colonizing human sebaceous glands (Nouveau-Richard *et al.*, 2007; Sakuma and Maibach, 2012).

Lipase is a key virulence factor secreted by *P. acnes*. This enzyme catalyzes the release of FFA from sebum triglycerides which trigger inflammatory reaction, adhesion and keratinocyte proliferation. Thus, the research of lipase inhibitor constitutes a relevant way to decrease inflammatory process and comedogenesis in sebaceous glands, and thus skin imperfections.

Whereas *P. acnes* is overexpressed in skin prone to acne, in oily skin without signs of acneic pimples, the bacterium is not overexpressed and consequently could not be counteracted by an antibacterial agent. In oily skin, it's more relevant to stop the virulence of the bacterium targeting its lipase activity in order to erase the imperfections and inflammation that can occur. To this end, we evaluated the potential of Bix'Activ to inhibit the activity of the lipase secreted by *P. acnes* without generating an antibacterial effect.

RESULTS & DISCUSSION

Bix'Activ did not modify the growth of *P. acnes* but it significantly inhibited ($p<0.001$) its lipase activity in a dose dependant manner, up to 68% at 0.02% (Figure 20).

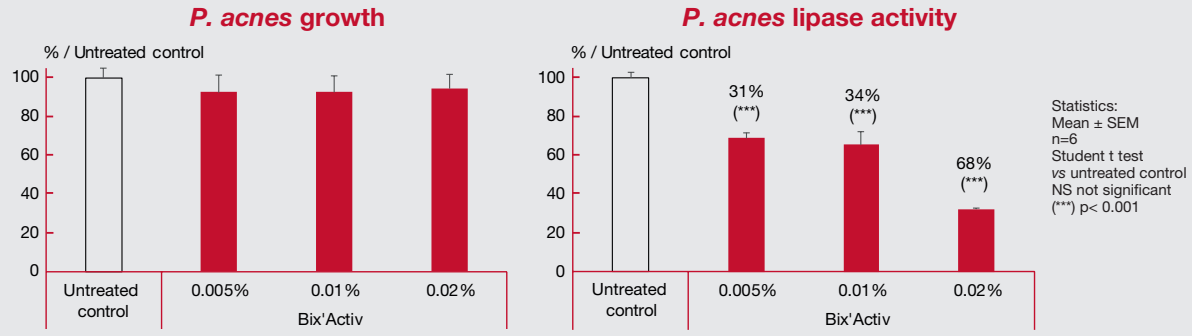


Figure 20 - Effect of Bix'Activ on *P. acnes* growth and lipase activity.

CONCLUSION

Bix'Activ inhibits *in vitro P. acnes* lipase activity and thus its virulence, without antibacterial effect, to avoid the worsening of the oily skin and the appearance of imperfections.

MATERIALS&METHODS

P. acnes (ATCC 6919 NCTC 737) are prepared at 10 million bacteria / mL in broth (trypic soy broth) medium with Bix'Activ at 0.005, 0.01 and 0.02%. After incubation for 3 days at 37°C in anoxia, the growth of the bacteria was measured by recording the optical density (OD) at 600 nm. Then the bacteria suspension was centrifuged (1500 g for 5 minutes) and lipase activity was determined by incubation of the supernatant with lipase substrate (1,2-Di-O-lauryl-rac-glycero-3-(glutaric acid 6-methylresorufin ester), Sigma, France) for 1 hour at 40°C. The lipase activity was evaluated by recording fluorescence emission at 600 nm with an excitation at 520 nm. The number of assays is n=6.

The results were expressed in % of control (basal medium) and presented as a mean ± standard deviation (SEM). The statistical analysis was done after normal distribution comparison of the values following SigmaPlot software recommendations (Systat Software Inc. USA). The threshold of significance was set to 5%.

Conclusions *in vitro*

We demonstrated the effects of Bix'Activ on:

- **Overproduction of lipids produced by sebocytes stimulated either by HS enriched in testosterone or by IGF-1**:
  • Asian donor: inhibition by 17%,
  • Black American donor: inhibition by 28%,
  • Caucasian donor: inhibition by 11%.

- **Hyperkeratinization of keratinocytes**:
  Activation of IGFBP-3 by 1.7 fold, to counteract IGF-1 action on keratinocytes.

- ***P. acnes* virulence**:
  Inhibition of *P. acnes* lipase activity by 68% without antibacterial effect.

EFFICACY

Improve the signs of Asian oily skin

in vivo

OBJECTIVE

In a double-blind, randomized, split-face, placebo-controlled clinical study, we evaluated the ability of Bix'Activ to improve the major signs of oily skin (Figure 21).

The study was performed on 35 Asian female subjects aged 20-45, with an oily skin (Lipidic index ≥ 120 measured with Sebumeter) and cheek sebaceous pore grade between 2 and 4, according to Bazin's atlas (Bazin R and Bazin F, 2010) Flament Skin Aging Atlas, Volume 2, Asian type, Editions Med'Com publishing 2010).

Bix'Activ at 0.25% and a placebo formulation were applied twice a day for a period of 56 days. The measurements were done at D0, D28 and D56.

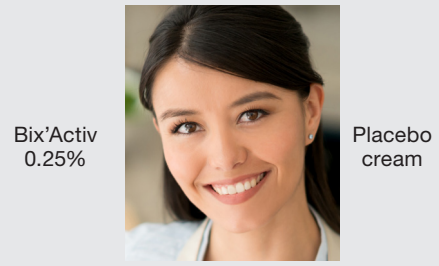


Figure 21 - Clinical study on oily skin signs with Bix'Activ *vs* Placebo:
- sebum secretion evaluation on the forehead (sebutape and image analysis and self assessment),
- skin pore evaluation on the cheeks (clinical scoring of sebaceous pores size, and self assessment),
- skin hydration (Corneometer and self assessment).

RESULTS & DISCUSSION

Reduced sebum production and resulting in skin shininess on the forehead

Figure 22 shows the spots representing the active sebaceous gland follicles of one volunteer. Therefore, the spots represent the sebum level and density secreted by the glands. We can see on the picture that Bix'Activ reduced the number and the size of the spots from D28 whereas the placebo showed no decrease from D0 to D56. The number of spots and area per spot (follicular sebum excretion rate in pixels) were calculated, compared at D28 and D56 to D0, and compared to the placebo formulation.

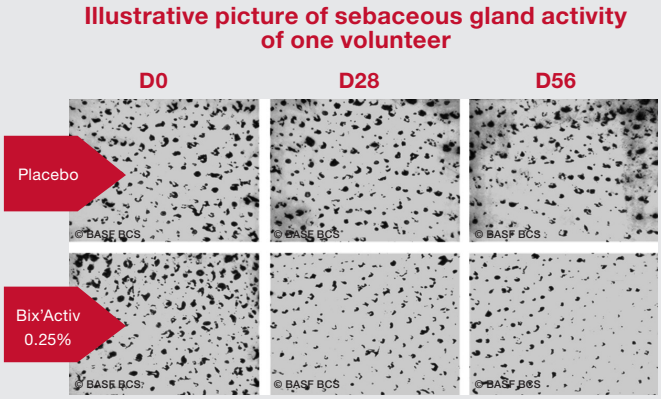


Figure 22 - Illustrative pictures of Sebutape of one volunteer. D0: before treatment, D28 and D56: after 28 and 56 days of product application.

Number of spots

After 28 days of treatment with Bix'Activ at 0.25%, the number of spots significantly decreased by 22% *vs* baseline ($p<0.001$) and 14% *vs* placebo ($p<0.01$). After 56 days of treatment, it significantly decreased by 37% *vs* baseline ($p<0.001$) and 25% *vs* placebo ($p<0.001$) (Figure 23).

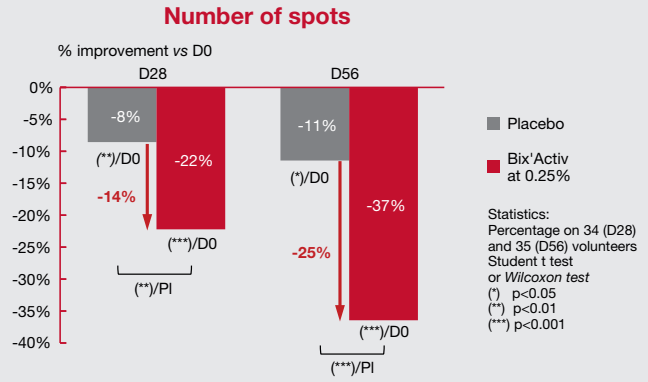


Figure 23 - Percentage of evolution of the number of spots on the forehead measured by image analysis on Sebutape.

in vivo

Area per spot

From 28 days of treatment with Bix'Activ at 0.25%, the area per spot decreased significantly by 46% *vs* baseline and 31% *vs* placebo. After 56 days of treatment, it decreased significantly by 62% *vs* baseline and 36% *vs* placebo (Figure 24).

Bix'Activ decreased both the number of spots and the area of spots showing a strong anti-seborrheic effect as early as 4 weeks.

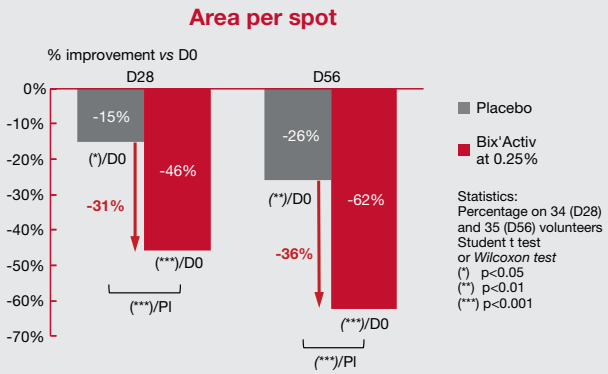


Figure 24 - Percentage of evolution of the area per spot on the forehead measured by image analysis on Sebutape.

Skin shininess reduction

A visible sign of oily skin related to this sebum overproduction is the skin shininess. Before/after pictures in Figure 25 illustrate the visible mattifying action of Bix'Activ.

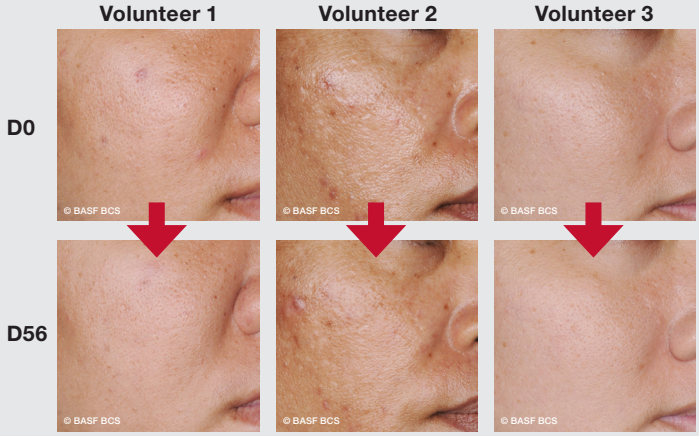


Figure 25 - Illustrative pictures of skin shininess, before (D0) and after treatment with Bix'Activ at 0.25% for 56 days from 3 volunteers.

Self-assessment

Moreover, the self-assessment, confirmed that Bix'Activ was visibly efficient on sebum reduction and on skin shininess reduction after 56 days of treatment. A significant majority of volunteers agreed on:

- **94%** The product reduces excessive sebum.
- **89%** I feel the product reduces skin oiliness and shininess.
- **91%** I feel the product goes deep in my pores for effective oil removal.
- **92%** I am feeling immediate matte feeling after use.
- **92%** This product controls oil production on my facial skin after use.
- **96%** My skin appeared healthier.

Sebaceous pore size reduction on the cheeks

After 28 days of treatment with Bix'Activ at 0.25%, the size of sebaceous pores decreased visibly and significantly by 3% *vs* baseline on the cheeks. After 56 days of treatment, the size of sebaceous pores decreased significantly by 9% *vs* baseline and by 3% *vs* placebo (Figure 26).

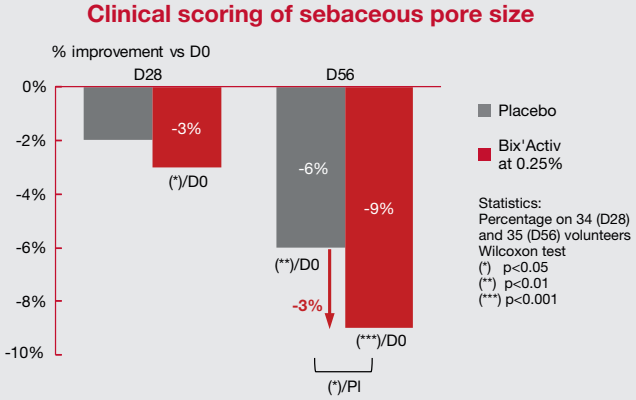


Figure 26 - Percentage of evolution of the clinical scoring of sebaceous pores on the cheeks.

Self-assessment

Moreover, the self-assessment, confirmed that Bix'Activ was visibly efficient reducing pore size after 56 days of treatment. A significant majority of volunteers agreed on:

- **86%** My skin pores look minimized after using the product.
- **94%** The product does not clog my skin's pores.
- **86%** The product tightens pores on my face.
- **88%** My pores appeared less visible.

in vivo

Imperfections reduction on the cheeks

Interestingly, we looked at the imperfections on the skin and we could see that Bix'Activ decreased the appearance of imperfections with visible improvement already after 4 weeks (Figure 27).

Reduced imperfections of one volunteer

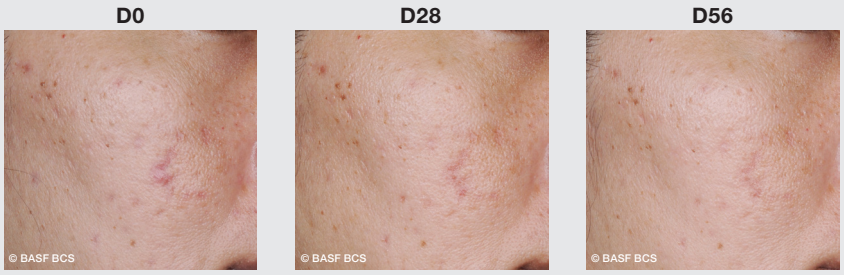


Figure 27 - Visible reduction of the imperfections of the skin of one volunteer.

Skin hydration is preserved

With the corneometer, we showed in figure 28 that Bix'Activ maintained skin hydration up to 56 days.

Hydration measurement

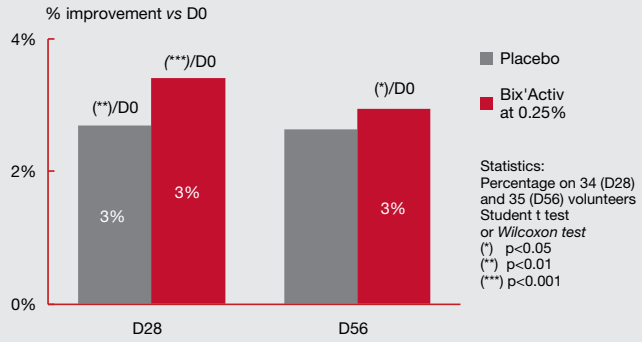


Figure 28 - Hydration measurement by Corneometer.

Self-assessment

Moreover, the self-assessment, confirmed that Bix'Activ preserved skin hydration. After 56 days of treatment, all volunteers agreed on:

100% My skin feels more moisturized.

100% This product leaves no feeling of tightness.

Bix'Activ keeps the skin moisturized, despite its strong efficacy on sebum regulation.

MATERIALS&METHODS

Study design

The clinical study was carried-out in double blind with randomization versus placebo. The efficacy of the formulation containing Bix'Activ at 0.25% was compared to the baseline (before treatment, D0) and to the half-face treated with the placebo formulation. The formulation is detailed in Annex 2. The study was conducted for a period of 56 days with check points at D0, D28 and D56.

Inclusion criteria

The study was done on 35 Asian females healthy volunteers, aged from 20 to 45, having lipidic index ≥120 µg/cm^2 on the face (sebumeter), and presenting cheek sebaceous pores grade between 2 and 4 (R. Bazin / F. Flament, Skin Aging Atlas).

Application modality

The products (a formulation containing Bix'Activ at 0.25% or a placebo formulation) were applied on the face by the volunteers twice a day on each half face for 56 days, under normal conditions of use.

Evaluation methods

Sebum secretion with Sebutape

Sebutape is a sebum-sensitive adhesive film. It allows the measurement of the sebum production. The evaluation of the sebaceous secretion with the Sebutape is perfectly suitable for assessing the effect of local treatments, regulating the physiology of the sebaceous glands.

The evaluation is carried out in an air-conditioned room (temperature: 22 ± 2°C, hygrometry: 50 ± 10%).The measurements must be imperatively made at the same time on each day of measurements (± 1hour).

The studied area is the forehead (two defined areas of left and right side of the forehead).

The skin on the forehead is first wiped three times with a cotton pad soaked with 2 ml of alcohol 70%, then dried with an absorbent paper. Tapes are sealed to the forehead skin, during a variable collection period (time necessary to reach a rank of 2 on the Sebutape scale) according to the degree of oiliness of the volunteer's skin. This time is noted at D0 for each volunteer and is used for the other measurement day (D28 and D56).

Image analysis is done using binary images of the sebum droplet distribution on the Sebutape.

For the analysis itself, the Sebutape is placed on a black, smooth and non-reflecting background. An image of each patch is taken with a Sony XC-711 CCD-RGB camera interfaced with a computer. The lighting, distance and focus conditions are constant.

Conversion to binary and threshold processes are carried out in order to obtain a black and white pattern.

Parameters and interpretation

- Number of spots (number of active sebaceous gland follicles).
- Area per spot (sebum excretion rate in pixels) = Total Spot Surface (Pixel) / Number of spots.

All these parameters must decrease for an anti-seborrheic effect.

Sebaceous pores with Clinical scoring

The trained evaluator in charge of the study performed visual clinical scores on the sebaceous pores before and after 28 and 56 days of application of the products using the scale on Bazin / F. Flament, Skin Aging Atlas, Asian type. This scale is composed of 6 grades from 0: no visible pores to 5: very visible pores.

The evaluation is carried out in a room under controlled temperature and relative humidity (temperature: 22 ± 2°C, hygrometry: 50 ± 10%).

Hydration with Corneometer

Corneometry is a technique used to determine the level of moisture in the outer layers of the stratum corneum. This method is based on the relationship between the electrical properties of skin tissues and their moisture content. A variation in the moisturization of the skin is traduced by a modification of the total capacitance of the system. The device used is a CM 825 (Courage and Khazaka, Germany).

The evaluation is carried out in a room under controlled temperature and relative humidity (temperature: 22 ± 2°C, hygrometry: 50 ± 10%).

The measurements are performed on both cheeks (Figure 29). The site of the instrumental measurements and their location at the different points of the kinetics should be strictly the most reproducible. The location is determined by using a cutaneous marking at T0.

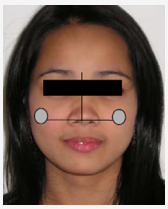


Figure 29 – Studied area by Corneometer CM 825.

Three measurements are performed on the study area for each volunteer and each examination time. Measurements are made at T0, T+4 weeks and T+8 weeks. All the measurements are performed by the same technician.

The mean of the 3 measurements is calculated for each area. The obtained results are expressed in arbitrary units. The increase in the parameter shows a moisturizing effect of the product.

Self-assessment

The volunteers are in front of a mirror and fill in the questionnaire individually without any extrinsic influences (other volunteers, results of technical measurements etc…). Questionnaires are filled in under the supervision of a technician to ensure that the questionnaire is correctly and completely filled in by the volunteers.

Statistics

a. Instrumental measurements

The statistical analysis of the evolution of the parameters in function of time were done after the verification of the normality of distribution using Shapiro-Wilk test.

Afterwards, the statistical analysis of the evolution of the studied parameters for each product was performed with the Student t test in case of the normality of distribution had been confirmed. In case of the distribution had not followed the normal law, a non-parametric test (Wilcoxon rank test) was used.

b. Clinical scoring

The statistical analysis of the evolution of the parameter has been done with non-parametric test: in function of time (Wilcoxon test) or in function of product (Mann-Whitney test).

c. Self-assessment

To evaluate the efficacy and the appreciation of the products for each item, two percentages Z1 and Z2 are calculated as follows:

-Z1 = favorable opinion ("completely agree" + "somewhat agree"),

-Z2 = unfavorable opinion ("completely disagree" + "somewhat disagree").

The statistical difference in frequencies (%) between favorable and unfavorable opinions is evaluated using Two-tailed binomial test at 5%.

Improve the signs of Black oily skin

in vivo

OBJECTIVE

In a double-blind, randomized, split-face, placebo-controlled clinical study, we evaluated the ability of Bix'Activ to improve the major signs of oily skin on black people (Figure 30).

The study was performed on 29 Black men and women volunteers (phototype V and VI) aged from 19 to 40 years old, with an oily skin (Lipidic index ≥ 100 µg/cm^2, measured with Sebumeter) and presenting a shiny skin.

Bix'Activ at 0.25% and a placebo formulation were applied twice a day for a period of 28 days. The measurements were done at D0 and D28.

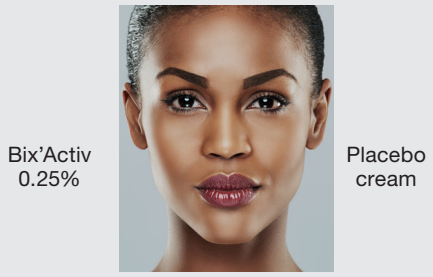


Figure 30 - Clinical study on the major signs of oily skin with Bix'Activ *vs* Placebo for 28 days:
- sebum secretion evaluation on the forehead (Sebumeter),
- skin shininess evaluation on the forehead (Glossymeter),
- skin hydration measurement on the cheeks (Corneometer).
Self-assessment was done on each studied parameter.

RESULTS & DISCUSSION

Reduced sebum secretion on the forehead

After 28 days of treatment with Bix'Activ at 0.25%, the quantity of excreted sebum at the skin surface decreased significantly by 44% versus baseline ($p<0.001$) and by 12% versus placebo ($p<0.05$) (Figure 31).

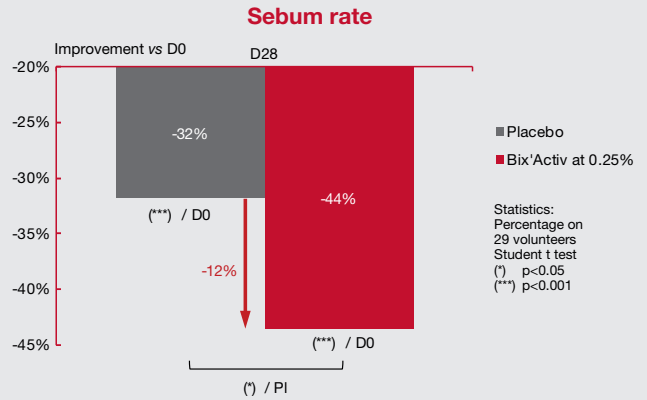


Figure 31 - Evolution of the percentage of the sebum rate (total mass of the lipids excreted by surface unit) on the forehead (Sebumeter).

Reduced skin shininess: mattifying effect

From 28 days of treatment with Bix'Activ at 0.25%, we observed a significant decrease of skin shininess by 11% *vs* baseline.

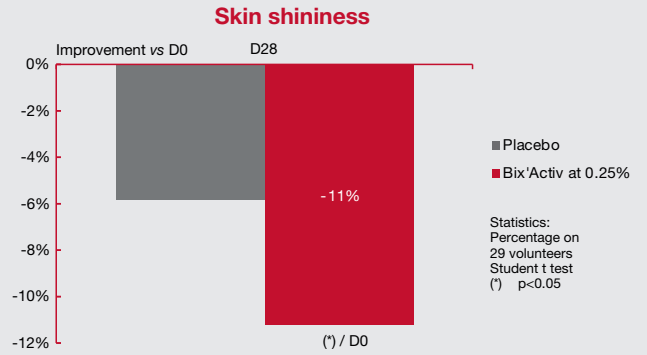


Figure 32 - Evolution of the percentage of the skin shininess (Glossymeter): mattifying effect of Bix'Activ.

in vivo

Illustrative pictures of skin shininess of three volunteers

Pictures in figure 33 illustrate the visible mattifying action of Bix'Activ after 28 days both on the cheeks and on the forehead. On the contrary, the placebo formula showed no significant effect on the skin shininess.

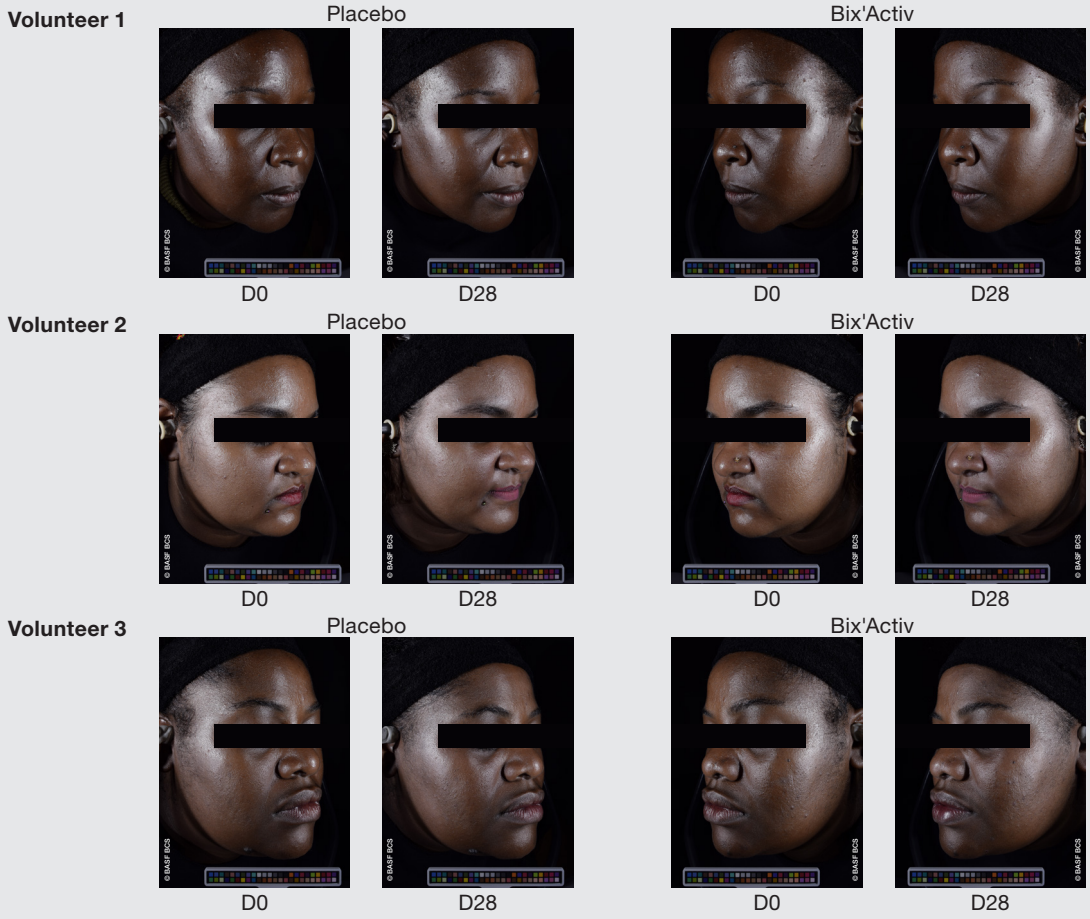


Figure 33 - Illustrative pictures of skin shininess, before (D0) and after treatment with Bix'Activ at 0.25% for 28 days on 3 volunteers.

Skin hydration is preserved

The measure done by the Cutometer revealed that Bix'Activ preserved the hydration level of the skin. With Bix'Activ, the skin dryness is kept at bay.

Self-assessment on Black oily skin

The self- assessment, confirmed that Bix'Activ was visibly efficient on the reduction of signs of oily skin. Bix'Activ was able to decrease skin shininess and to improve the general aspect of the skin after 28 days of treatment. including reduction of pore size and imperfections. A significant majority of volunteers agreed on:

- **93%** The skin is less oily (less sebum).
- **90%** The pores are tightened.
- **86%** The complexion is radiant.
- **90%** The skin is clear.
- **90%** Skin is healthier (less imperfections).
- **79%** The skin is more matte.
- **86%** The skin texture is refined.
- **97%** The skin is hydrated, not dried
- **90%** Imperfections are reduced.

MATERIALS&METHODS

Study design

The clinical study was carried-out in double blind with randomization versus placebo. The efficacy of the formulation containing Bix'Activ at 0.25% was compared to the baseline (before treatment, D0) and to the half-face treated with the placebo formulation. The formulation is detailed in Annex 2. The study was conducted for a period of 28 days with check points at D0, and D28.

Inclusion criteria

The study was done on 29 Black male and female healthy volunteers (phototype V and VI), aged from 19 to 40, having lipidic index ≥100 µg/cm^2 on the face (Sebumeter), and presenting shiny skin.

Application modality

The products (a formulation containing Bix'Activ at 0.25% or a placebo formulation) were applied on the face by the volunteers twice a day on each half face for 28 days, under normal conditions of use.

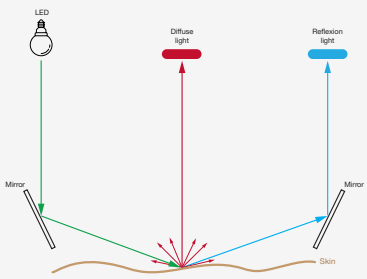
Evaluation methods

Sebum secretion (Sebumeter)

Sebumeter is a photometric method. A synthetic ribbon, which becomes transparent when in contact with absorbed lipids, is applied to the measurement zone for precisely 30 seconds. Its transparency increases proportionally with the quantity of sebum from the hydrolipidic film with which it is in contact. A reflectometry recording is used to quantify the increase of the light transmitted and to determine the total mass of the lipids excreted by the surface unit (in µg/cm^2).
The evaluation is carried out in an air-conditioned room (temperature: 22 ± 2°C, hygrometry: 50 ± 10%) The measurements must be imperatively made at the same time on each day of measurements (± 1hour).
The studied area is the forehead (two defined areas of left and right side of the forehead).
The sebum rate is calculated with the total mass of the lipids excreted by the surface unit (in µg/cm^2). A decrease of the sebum rate corresponds to oil reduction.

Mattifying effect (Glossymeter)

The Skin-Glossymeter GL200 (Courage & Khazaka) measures both the fraction of light being reflected directly from the sample (skin) and the scattered fraction of reflected light. The fraction of directly reflected light is correlated with the gloss.
When the probe is in contact with the measurement zone, white light is emitted from a LED on a mirror which reflects the light at an angle of 60° on the sample (green arrow).
The sample reflects one fraction of the emitted white light at the same angle which is captured in the blues sensor after having been reflected by another mirror (blue arrow).
At the same time the sample deflects another fraction of the LED light diffusively. This fraction of diffuse light reflection is captured by another sensor of the device (red arrow).
The probe, 2.5 x 5 mm, is especially designed to assess the gloss of small surface.



DSC (Diffuse Scattering Correction) technology of the Skin-Glossymeter GL200 allows to compare the gloss assessment of different types of skin /hair/nails/lips.

The evaluation is carried out in a room under controlled temperature and relative humidity (temperature: 22 ± 2°C, hygrometry: 50 ± 10%).
The studied area is the forehead (two defined areas of left and right side of the forehead).
Values are expressed in "Glossymeter Units" (GU), based on DIN and ISO norms.
A decrease of GU corresponds to a decrease of shininess and consequently to a mattifying effect.

Hydration (Corneometer)

Cutaneous hydration measurements are performed with a Corneometer CM 825 (Courage & Khazaka). The measuring principle is based on capacitance measurement. The surface of the measurement head, in contact with the skin, modifies its electrical capacity according to the humidity level of the skin.
This technique is a well-established method to reproducibly and accurately determine the hydration level of the skin surface, i.e. the humidity level of the most external cutaneous layers of the Stratum Corneum (10-20 µm depth).

Illustrative photographs (Colorface)

The ColorFace acquisition system is a dedicated solution for standardized imaging in the clinical study setting for evaluation of a clinical effect or for a screening phase. This innovative solution is based on a high-resolution sensor to obtain high quality images.

Self-assessment

The volunteers are in front of a mirror and fill in the questionnaire individually without any extrinsic influences (other volunteers, results of technical measurements etc…). Questionnaires are filled in under the supervision of a technician to ensure that the questionnaire is correctly and completely filled in by the volunteers.

Principle

Questionnaires are filled in under the supervision of a technician to ensure that the questionnaire is correctly and completely filled in by the subject.
For each Item, the possible answers are:
- 1: completely agree,
- 2: somewhat agree,
- 3: somewhat disagree,
- 4: completely disagree.

The evaluation was performed on face.
The analysis involves establishing frequency tables that take into account the number of responses and calculate the frequency of the different possible answers (given as percentage) to each qualitative question.
To evaluate the efficacy and the appreciation of the products for each item, two percentages Z1 and Z2 are calculated as follows:
- Z1 = favorable opinion (ex: "Totally agree" + "Agree"),
- Z2 = unfavorable opinion (ex: "Disagree" + "Totally disagree").

The statistical difference in frequencies (%) between favorable and unfavorable opinions is evaluated using Two-tailed binomial test at 5%.

Statistical method

The statistical analysis of the evolution of the parameters in function of time were done after the verification of the normality of distribution using Shapiro-Wilk test.
Afterwards, the statistical analysis of the evolution of the studied parameters for each product was performed with the Student t test in case of the normality of distribution had been confirmed. In case of the distribution had not followed the normal law, a non-parametric test (Wilcoxon rank test) was used.

Conclusions *in vivo*

In vivo, Bix'Activ demonstrated efficacy on signs of oily skin on multiple skin types:

Asian population

- sebum production is reduced vs baseline and *vs* Placebo (number and area of spots),
- shininess of the skin is reduced,
- pore size is reduced by 9% vs baseline,
- visible imperfections are decreased,
- skin hydration is preserved.

Black population

- sebum production is significantly reduced both *vs* baseline and *vs* Placebo.
- shininess is significantly reduced versus baseline,
- skin hydration is preserved.

All these results were confirmed by a self-assessment.

In general, Bix'Activ at 0.25% showed its capacity to improve the appearance of oily skin and helps recover a mattified and healthy skin aspect.

GENERAL CONCLUSION

Bix'Activ is the cutaneous diet that mattifies and beautifies the skin. Bix'Activ is a multi-ethnic solution with visible effects on skin shininess by slowing-down sebum production. Bix'Activ is also a solution to reduce the size of pores and to reduce skin blemishes.

Concerns about oily skin affect everyone worldwide, regardless of ethnicity, age or gender. Three clinical signs are linked to oily skin: skin shininess, enlarged pores and skin imperfections.

We developed a standardized extract of *Bixa orellana* seeds, Bix'Activ, to correct these three main signs of oily skin.

We demonstrated that Bix'Activ is able to reduce the production of lipids in normal human primary sebocytes (in 2D and in a new 3D sebaceous gland model). The reduction was observed regardless of the ethnicity of sebocytes and through two signaling pathways inducing sebum overproduction: androgen pathway through human serum and IGF-1 pathways, respectively reported to be stimulated by hormonal changes and sugar- or dairy-rich diets.

Bix'Activ is also efficient in reducing keratinocyte hyperkeratinization *in vitro*, by targeting IGFBP3, a specific component of the IGF-1 signaling pathway. It also inhibits the virulence of *P. acnes in vitro*, by decreasing lipase activity.

Tested *in vivo*, on Asian and Black population in placebo-controlled studies, Bix'Activ clearly mattifies the skin with a significant inhibition of sebum production and a constant level of skin moisturization on both populations. Additionally, Bix'Activ decreases pore size and imperfections in Asian population.

Bix'Activ is a multi-ethnic solution to get a fresh and healthy appearance.

ANNEXES

Annex 1 - Technical data - Available upon request

- Quality and Regulatory Product Information
- Composition sheet
- Specifications
- Formulation Data Sheet

Annex 2 - Clinical test formula

For the test done in Asia

Trade name	INCI name	Placebo formulation %	Bix'Activ BC10050 formulation %
Emulgin SG	Sodium Stearoyl Glutamate	0.50	0.50
Citric acid solution 10%	Citric Acid (and) Water	0.70	0.65
Cosmedia SP	Sodium Polyacrylate	0.70	0.70
Cutina PES	Pentaerythrityl Distearate	1.00	1.00
Glycerine 99-5	Glycerin	2.00	2.00
Elestab 388	Propylene Glycol (and) Phenoxyethanol (and) Chlorphenesin (and) Methylparaben	2.50	2.50
Emulgade Sucro Plus	Sucrose Polystearate (and) Cetyl Palmitate	3.00	3.00
Myritol 318	Caprylic/ Caprylic Triglyceride	3.00	3.00
Cetiol C 5 C	Coco-Caprylate / Caprate	3.00	3.00
Cetiol CC	Dicaprylyl Carbonate	3.00	3.00
Bix'Activ BC10050	Maltodextrin (and) Bixa Orellana Seed Extract	-	0.25
Water	Aqua	qsf 100	qsf 100

For the test done in Africa

Trade name	INCI name	Placebo formulation %	Bix'Activ BC10113B formulation %
Emulgin 388	Propylene Glycol (and) Phenoxyethanol (and) Chlorphenesin (and) Methylparaben	2.50	2.50
Glycerine 99-5	Glycerin	2.00	2.00
Cosmedia ACE	Sodium Polyacrylate (and) Dicaprylyl Carbonate (and) Polyglyceryl-3 Caprate	2.00	2.00
Cetiol 4 All	Dipropylheptyl Carbonate	5.00	5.00
Cetiol® CC	Dicaprylyl Carbonate	3.00	3.00
Bix'Activ BC10050	Maltodextrin (and) Bixa Orellana Seed Extract	-	0.25
Water	Aqua	qsf 100	qsf 100

Annex 3 - Formulation examples

Mattifying Cream (SC-FR-18-BC-50802-07)

Phase	Ingredients	INCI	% by weight	Function
A	Emulgade® Sucro Plus	Sucrose Polystearate, Cetyl Palmitate	3.00	Emulsifier (O/W)
	Cutina® PES	Pentaerythrityl Distearate	1.00	Consistency agent
	Myritol® 318	Caprylic/Capric Triglyceride	3.00	Emollient
	Cetiol® C 5C	Coco-Caprylate/Caprate	3.00	Emollient
	Cetiol® CC	Dicaprylyl Carbonate	3.00	Emollient
	Cosmedia® SP	Sodium Polyacrylate	0.70	Rheology modifier
B	Water, demin.	Aqua	80.90	
	Glycerin	Glycerin	2.00	Humectant
	Eumulgin® SG	Sodium Stearoyl Glutamate	0.50	Emulsifier (O/W)
	Preservative*		qs	Preservative
C	Bix'Activ™ BC10050	Bixa Orellana Seed Extract, Maltodextrin	0.25	Active ingredient
	Water, demin.	Aqua	2.00	
D	Perfume*	Parfum	q.s.	Fragrance
E	Citric Acid (10% solution)	Citric Acid	0.65	pH Adjustment

Specifications	
pH value (23°C)	6.20

Pore Refining Toner (SC-FR-18-BC-50848-01)

Phase	Ingredients	INCI	% by weight	Function
A	Water, demin.	Aqua	87.65	
	Glycerin	Glycerin	4.00	Humectant
	EDTA® BD	Disodium EDTA	0.05	Complexing agent
	Euxyl K 712 *(Schülke)*	Aqua, Sodium Benzoate, Potassium Sorbate	1.00	Preservative
	Eumulgin® SML 20	Polysorbate 20	1.00	Emulsifier (O/W)
	Pluracare® L 64 G	Poloxamer 184	2.00	Surfactant
	Zinc Gluconate *(Corbion)*	Zinc Gluconate	0.05	Active ingredient
B	Water, demin.	Aqua	3.00	
	Bix'Activ™ BC10050	Bixa Orellana Seed Extract, Maltodextrin	0.25	Active ingredient
	Citric Acid (10% solution)	Citric Acid	0.40	pH Adjustment
C	Cetiol® HE	PEG-7 Glyceryl Cocoate	0.50	Emollient
	Perfume*	Parfum	0.10	Fragrance

Specifications	
pH value (23°C)	5.10

BIBLIOGRAPHY

Akamatsu H, Zouboulis CC, Orfanos CE.
Control of human sebocyte proliferation *in vitro* by testosterone and 5-alpha-dihydrotestosterone is dependent on the localization of the sebaceous glands.
J Invest Dermatol, 99: 509–511, 1992

Barreca A, De Luca M, Del Monte P, Bondanza S, Damonte G, Cariola G, Di Marco E, Giordano G, Cancedda R, Minuto F.
In vitro paracrine regulation of human keratinocyte growth by fibroblast-derived insulin-like growth factors.
J Cell Physiol 151:262–8, 1992

Bazin R and Bazin F.
Flament Skin Aging Atlas, Volume 2, Asian type.
Editions Med'Com, 2010

Bickers DR, Lim HW, Margolis D, Weinstock MA, Goodman C, Faulkner E et al.
The burden of skin diseases
Journal of the American Academy of Dermatology 55:490-500, 2006

Cappel M, Mauger D, Thiboutot D.
Correlation between serum levels of insulin-like growth factor 1, dehydroepiandrosterone sulfate, and dihydrotestosterone and acne lesion counts in adult women.
Arch Dermatol 141:333–8, 2005

Choi EH, Man MQ, Wang F, Zhang X, Brown BE, Feingold KR, Elias PM.
Is endogenous glycerol a determinant of stratum corneum hydration in humans?
J Invest Dermatol. 125:288–293, 2005

Epstein EH, Epstein WL.
New cell formation in human sebaceous glands.
J Invest Dermatol. 46(5):453-8, 1966

Feingold KR.
The importance of lipids in cutaneous function.
J Lipid Res. 48(12):2529-30, 2007

Goh CL, Noppakun N, Micali G, Azizan NZ, Boonchai W, Chan Y, Cheong WK, Chi Chiu P, Etnawati K,8 Gulmatico-Flores Z, Foong H, Kubba R, Paz-Lao P, Lee YY, Loo S, Modi F, Nguyen TH, Pham TL, Shih YH, Sitohang IB, Wong SN
Meeting the Challenges of Acne Treatment in Asian Patients: A Review of the Role of Dermocosmetics as Adjunctive Therapy
J Cutan Aesthet Surg. 9(2): 85–92, 2016

Higaki S, Kitagawa T, Kagoura M, Morohashi M, Yamagishi T.
Correlation between Propionibacterium acnes biotypes, lipase activity and rash degree in acne patients.
J Dermatol. 27(8):519-22, 2000

Hillebrand GG, Levine MJ, Miyamoto K.
The Age-Dependent Changes in Skin Condition in African Americans, Asian Indians, Caucasians, East Asians, and Latinos.
IFSCC Magazine, Vol 4, N° 4, 2001

Isard O, Knol AC, Ariès MF, Nguyen JM, Khammari A, Castex-Rizzi N and Dréno B.
Propionibacterium acnes activates the IGF-1/IGF-1R system in the epidermis and induced keratinocyte proliferation.
J. Invest. Dermatol. 131:59-66, 2011.

Jacobsen E, Billings JK, Frantz RA, Kinney CK, Stewart ME, Downing DT.
Age-related changes in sebaceous wax ester secretion rates in men and women.
J Invest Dermatol. 85: 483–485, 1985

Jarrousse, V, Castex-Rizzi, N, Khammari, A, Charveron, M, Dréno, B.
Modulation of integrins and filaggrin expression by Propionibacterium acnes extracts on keratinocytes.
Archives of Dermatological Research 299(9), 441-44, 2007

Jung Y.R, Lee J.H, Sohn K.C, Lee Y, Seo Y.J, Kim C.D, Lee J.H, Hong S.P, Seo S.J, Kim S.J, and Myung I.
Adiponectin Signaling Regulates Lipid Production in Human Sebocytes.
PLOS one 12(1), 2017

Krane JF, Gottlieb AB, Carter DM, Krueger JG.
The insulin like growth factor I receptor is over-expressed in psoriatic epidermis, but is differentially regulated from the epidermal growth factor receptor.
J Exp Med. 175:1081–1090, 1992

Liu J, Yan R, Zhong Q, Ngo S, Bangayan NJ, Nguyen L, Lui T, Liu M, Erfe MC, Craft N, Tomida S, Li H.
The diversity and host interactions of Propionibacterium acnes bacteriophages on human skin.
ISME J. Sep;9(9):2078-93, 2015

Melnik BC, Schmitz G.
Role of insulin, insulin-like growth factor-1, hyperglycaemic food and milk consumption in the pathogenesis of acne vulgaris.
Exp Dermatol.18(10):833–841, 2009

Melnik.
Dietary intervention in acne. Attenuation of increased mTORC1 signaling promoted by Western diet.
Dermato- Endocrinology 4:1, 20-32, 2012

Nouveau-Richard S, Zhu W, Li YH, Zhang YZ, Yang FZ, Yang ZL, Lian S, Qian BY, Ran YP, Bouillon C, Chen HD, de Lacharrière O.
Oily skin: specific features in Chinese women.
Skin Res Technol. 13(1):43-8, 2007

Ottaviani M, Alestas T, Flori E, Mastrofrancesco A, Zouboulis CC, Picardo M.
Peroxidated squalene induces the production of inflammatory mediators in HaCaT keratinocytes: a possible role in acne vulgaris.
J Invest Dermatol. 126(11):2430-7, 2006

Pappas A, Fantasia J, Chen T.
Age and ethnic variations in sebaceous lipids.
Dermato-Endocrinology 5:2, 319-324, 2013

Picardo M, Monica Ottaviani, Emanuela Camera, and Arianna Mastrofrancesco.
Sebaceous gland lipids.
Dermatoendocrinol. 1(2): 68–71, 2009

Richard M.A, Corgibet F, Dupin N, Chaussade V, Philippe C, Taieb C, Joly P, Ezzedine K, Misery L.
La peau des Français. Analyse des caractéristiques de notre peau à partir de l'étude Objectifs Peau.
Annales de Dermatologie et de Vénéréologie. 144, 12, S108-S109, 2017

Sadagurski M, Yakar S, Weingarten G et al.
Insulin-like growth factor 1 receptor signaling regulates skin development and inhibits skin keratinocyte differentiation.
Mol Cell Biol. 26:2675–87, 2006

Sakuma TH, Maibach HI.
Oily skin: an overview.
Skin Pharmacol Physiol. 25(5):227-35, 2012

Shi VY, Leo M, Hassoun L, Chahal DS, Maibach HI, Sivamani RK.
Role of sebaceous glands in inflammatory dermatoses.
J Am Acad Dermatol. 73:856–863, 2015

Smith RN, Mann NJ, Braue A, Mäkeläinen H, Varigos GA.
The effect of a high-protein, low glycemic-load diet versus a conventional, high glycemic-load diet on biochemical parameters associated with acne vulgaris: a randomized, investigator-masked, controlled trial.
J Am Acad Dermatol 57: 247–256, 2007

Smith TM, Gilliland K, Clawson GA, Thiboutot D.
IGF-1 induces SREBP-1 expression and lipogenesis in SEB-1 sebocytes via activation of the phosphoinositide 3-kinase/Akt pathway.
J Invest Dermatol.128: 1286–1293, 2008

Smith KR, Thiboutot DM.
Thematic review series: skin lipids. Sebaceous gland lipids: friend or foe?
J Lipid Res. 49(2):271-81, 2008

Tavakkol A, Elder JT, Griffiths CEM, Cooper KD, Talwar H, Fisher GJ, Keane KM, Foltin SK, Voorhees JJ.
Expression of growth hormone receptor, insulin-like growth factor I (IGF-I) and IGF-I receptor mRNA and proteins in human skin.
J Invest Dermatol 99:343–349, 1992

Vora S, Ovhal A, Jerajani H et al.
Correlation of facial sebum to serum insulin-like growth factor-1 in patients with acne.
Br J Dermatol 159:990–1, 2008

Xia L, Zouboulis CC, Ju Q.
Culture of human sebocytes in vitro
Dermatoendocrinol. 1(2):92-5, 2009

Xu F, Yan S, Wu M, et al.
Ambient ozone pollution as a risk factor for skin disorders.
Br J Dermatol. 165(1):224–225, 2011

Zouboulis CC, Xia L, Akamatsu H, Seltmann H, Fritsch M, Hornemann S, Rühl R,Chen W, Nau H, Orfanos CE.
The human sebocytes culture model provides new insights into development and management of seborrhea and acne.
Dermatology, 196: 21– 31, 1998

Europe

BASF Beauty Creations

49, avenue Georges Pompidou
92593 Levallois-Perret Cedex
France

Phone: +33 (0) 1 49 64 52 09
bcs-europe@basf.com

North America

BASF Corporation
North American Regional Headquarters
100 Park Avenue
Florham Park, NJ 07932
USA

Phone: +1-800-962-7831
CosmeticsCustomerCare@basf.com

South America

BASF S.A.
Av. das Nações Unidas, 14171
Crystal Tower
04794-000 - São Paulo - SP
Brazil

Phone: +55 11 2039 2273
personal-care-sa@basf.com

Asia Pacific

BASF East Asia
Personal Care
45/F Jardine House
No.1 Connaught Place
Central, Hong Kong

Phone: +852 2731 0190
personal-care-hk@basf.com

Publisher
BASF Beauty Care Solutions France SAS
3, Rue de Seichamps - 54425 Pulnoy (France)
Email: bcs-europe@basf.com
www.carecreations.basf.com

Pictures

Global Edition, Sept. 2018 (update July 2019)