PRISTINE MECHANICAL EXTRACTION

Active ingredients in Pure Guild Moisturizing Treatment for Ultra-Dry Skin maintain their molecular integrity and full spectrum of biological activities because no solvents are used and no heat is applied to extract them.

Pure Guild employs only gentle mechanical compression over heat to render highly effective compounds from potent raw materials. Although costly, this process yields a superior molecule, while other organic brands use chemical solvents like hexane or ether, which adulterate the final product, and heat distillation, which inhibits therapeutic properties. Super-premium Pure Guild cosmeceuticals contain no sodium lauryl sulfate or other detergents. They are strictly hypoallergenic, non-irritating, and never tested on animals.

BREAKING SCIENCE FOR ULTRA-DRY SKIN

While common moisturizers dissipate quickly, advanced Pure Guild Moisturizing Treatment strengthens and rebuilds the barrier function of the stratum corneum to retain innate moisture and resist environmental assault. Pure Guild achieves such intense moisturization because its active ingredient, *Pichia anomala* extract, stimulates lamellar-lipid transport, synthesis, and maturation within the epidermis.

» *Pichia anomala* recruits exogenous lipids into the keratinocytes to render highly effective compounds from potent raw materials. Although costly, this process yields a superior molecule, while other organic brands use chemical solvents like hexane or ether, which adulterate the final product, and heat distillation, which inhibits therapeutic properties. Super-premium Pure Guild cosmeceuticals contain no sodium lauryl sulfate or other detergents. They are strictly hypoallergenic, non-irritating, and never tested on animals.

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PINNACLE OF PURITY IN BIOTECHNOLOGY

Pure Guild Moisturizing Treatment for Ultra-Dry Skin sets the new benchmark for molecular purity in topical treatments:

» Pure Guild extracts pristine active compounds mechanically, using no industrial solvents or damaging heat, creating a standard of purity by which other products can be measured.

» Pure Guild employs only the active ingredients proven most effective in rigorous clinical trials, creating a standard of cosmeceutical performance.

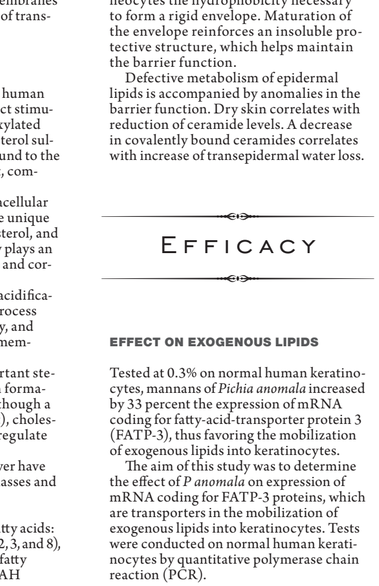

Figure 2. Extreme sun damage, one indication for Pure Guild Moisturizing Treatment for Ultra-Dry Skin

Replenishing the Lipids for Skin Moisture

A disruption in the lipid structure of the stratum corneum induces dynamic regeneration in the epidermis to ensure homeostasis of the skin's barrier function.

That is why Pure Guild Moisturizing Treatment for Ultra-Dry Skin incorporates *Pichia anomala* extract, a novel active botanical that boosts the synthesis, transport, secretion, and maturation of epidermal lipids. *P anomala* extract encourages formation of a functional lipid cement by stimulating:

» Recruitment of exogenous lipids,
» Transportation and secretion of precursor lipids via lamellar bodies, and
» Maturation of lipid precursors into functional lipids in the lamellar layers and the cornified envelope.

A pure mannan fraction obtained from *Pichia anomala* is a high-technology active ingredient — a result of advanced research on fermentation. It boosts the natural lipid replenishment system of the skin to reinforce the integrity of its barrier function.

BARRIER FUNCTION OF THE EPIDERMIS

The stratum corneum is made of corneocytes, which are keratinocytes cemented together by lamellar-lipid membranes. These lipids play a fundamental role in the structure and function of the epidermis, forming a barrier.

Their composition — 50 percent ceramides, 25 percent cholesterol, and 15 percent long-chain free fatty acids — and their arrangement as lipid bilayers in the spaces between the corneocytes determine the characteristics of the barrier.

The process of synthesis, transportation, secretion, and maturation of epidermal lipids ensures the homeostasis of the barrier function. The production of lamellar bodies, which release their contents into the intercellular spaces, continuously regenerates the lipid cement of the skin.

Epidermal lipid flow has several steps. First, lipids are synthesized by skin cells in the form of polar precursors — glucosylceramides, cholesterol, and phospholipids — from metabolic intermediates and fatty acids. These fatty acids may come from extra-cutaneous sites and be internalized by keratinocytes via specialized membrane transporters such as fatty-acid-transporter proteins (FATP).

The lipid precursors are generated in quantity and stored in organelles known as lamellar bodies, formed from the Golgi apparatuses. Lamellar bodies appear in the upper spiny layer, increasing volume and size in the granular layer. Inclusion of lipids within the lamellar bodies is ensured by ABCA12 proteins on their membranes. Lamellar bodies also contain lipases for lipid maturation, proteases and protease inhibitors for regulating desquamation, structural components for the corneal envelope, and anti-microbial peptides.

Lamellar bodies fuse with plasma membranes in the terminal differentiation phase and secrete their contents into the intercellular spaces at the junction of the stratum granulosum and the stratum corneum. Lipid precursors undergo modification by the enzymes β-glucocerebrosidase, phospholipase, and sphingomyelinase, released at the same time.

Modification of the polarity and structure of the precursors results in the formation of lamellar membranes, the composition and arrangement of which determine the characteristics of the barrier function.

Omega-hydroxyceramides are bound to the envelopes of corneocytes, anchoring them to the lamellar membranes.

The essential function of the stratum corneum — barrier — has been known for years. Any quantitative or qualitative anomaly in its lipids leads to an increase of transepidermal water loss, a reliable marker. Many active ingredients have been proposed in recent years to encourage synthesis of new epidermal lipids to restructure the skin barrier.

Any disruption of the intercellular lipid cement, caused by solvents like acetone, detergents like sodium lauryl sulfate, or other caustic chemicals, provokes a rapid response in the skin:

» Secretion of the lamellar bodies already present in keratinocytes of the stratum granulosum (0–30 minutes),
» Increase in lipid synthesis (30–60 minutes),
» Formation of new lamellar bodies and exocytosis of their contents to replenish intercellular spaces (30 minutes to six hours), and
» Enzymatic modification of precursor lipids into mature, functional lipids.

Pure Guild Moisturizing Treatment represents a new generation of active botanicals capable of stimulating the key steps to forming lipid cement:

» Recruitment of exogenous lipids into keratinocytes by means of specific transporters,
» Traffic and secretion of lipid precursors via lamellar bodies, and
» Maturation of lipid precursors into the functional lipids of the lamellar layers in the cornified envelope.

Pure Guild Moisturizing Treatment, developed from the yeast *Pichia anomala*, also used in food processing, contains pure, natural polysaccharides from mannans, which boost epidermal lipid flow to restore the skin's barrier function.

RECRUITMENT OF LIPIDS

Tested at 0.3% on normal human keratinocytes, *Pichia anomala* extract stimulates by 33 percent the expression of fatty-acid-transporter protein 3 (FATP-3),

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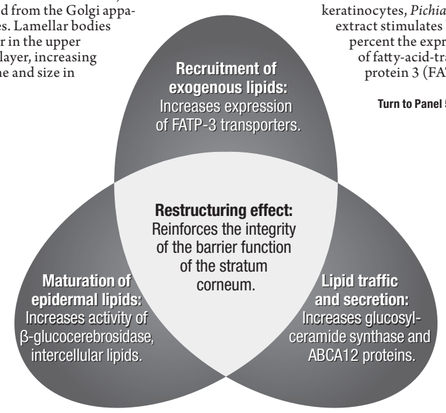

Tested in vivo after 14 days of twice-daily treatment, Pichia anomala *extract formulated at 3% reduced transepidermal water loss by 14 percent, compared to a placebo, even after repeated aggression by sodium lauryl sulfate.* P anomala *extract restructured the lipid cement of the stratum corneum and reinforced the integrity of the barrier function.*

which is involved in the internalization of exogenous lipids by the keratinocytes.

Precursors of epidermal lipids are formed in Golgi apparatuses of granular keratinocytes, either by neo-synthesis or by derivation from fatty acids of extracutaneous origin. To internalize the fatty acids, keratinocytes have FATPs, specialized membrane proteins for transporting lipids through their plasma membranes.

The FATP family has six homologous members, FATP-1 to FATP-6, in various tissues that metabolize or store fatty acids.

These transmembrane proteins have a highly conserved sequence of 311 amino acids, involved in active transport of long-chain and very-long-chain fatty acids across the keratinocyte-plasma membrane. Fatty-acid recruitment is correlated directly with the level of FATP expression.

FATP-related fatty-acid transport may be linked to acyl-CoA synthetase activity. Exogenous fatty acids transferred to the intracellular space by FATPs are routed to various organelles where they are metabolized to synthesize lipid precursors.

SECRETION OF LIPIDS

Lipid traffic within keratinocytes is conducted by lamellar bodies, composed mostly of glucosylceramides. It is a complex process of several steps:

» Ceramide neo-synthesis from fatty acids and serine, which takes place in the endoplasmic reticulum,
» Ceramide glucosylation to form glucosylceramides, catalyzed in the Golgi apparatus by glucosylceramide synthase.
» Precursor transfer to lamellar bodies and secretion via ABCA12 proteins.

GLUCOSYLCERAMIDE SYNTHASE

Tested at 0.3% on normal human keratinocytes, *Pichia anomala* extract stimulates by 22 percent the expression of glucosylceramide synthase, involved in the synthesis of the precursors of most ceramides.

Ceramides are major elements of the inter-corneocyte-lipid matrix and are involved in stress responses such as differentiation, apoptosis, and senescence.

The transport of glucosylated ceramides in lamellar bodies protects the cytosol of granular keratinocytes from premature apoptosis by sequestrating ceramides as glucosylceramides, a non-toxic form.

Ceramides are converted into glucosylceramides by glucosylceramide synthase in the Golgi apparatuses — an activity that increases during keratinocyte differentiation and regulates the specialized production of sphingolipids.

Glucosylceramides are the precursors of more than 60 percent of total epidermal ceramides, including at least five different types. Temporary formation of glucosylceramides also determines the formation of lamellar bodies, the metabolism of ceramides, and their arrangement in the lipid matrix. A deficiency of glucosylceramide synthase disrupts the barrier function of skin.

EXPRESSION OF PROTEINS

Tested at 0.3% on normal human keratinocytes, *Pichia anomala* extract stimulates by 25 percent the expression of ABCA12 proteins involved in formation of lamellar bodies and the secretion of their contents.

After synthesis in the Golgi apparatus, a polar lipid precursor is transferred to a lamellar body by means of an ABCA12 protein, which enables transport of molecules across cell membranes. The ABCA subclass is specialized for transport of endogenous lipids.

ABCA12 proteins are expressed in the granular cells of the epidermis and located on lamellar body membranes. They play an important role in the regulation of intercellular traffic and the secretion of precursor lipids. Lamellar bodies with ABCA12 on their membranes fuse with the apical plasma membranes of keratinocytes and release their contents into the intercellular spaces. ABCA12 protein expression is regulated by the peroxisome-proliferator-activated receptor and the liver-X receptor.

An ABCA12 gene mutation, responsible for ichthyosis and other effects, alters lipid transport and secretion, blocking flow. Lamellar body formation becomes abnormal, and synthesis, distribution, and secretion of lipids become defective.

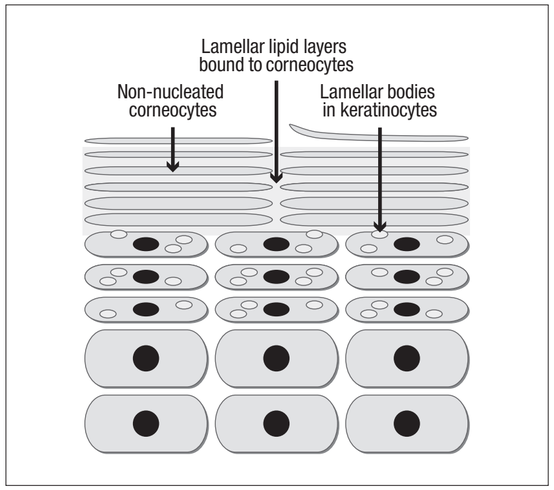

Figure 1. Lamellar lipid layers, formed from lamellar bodies within keratinocytes, cementing non-nucleated corneocytes to create a functional barrier

B-GLUCOCERE-BROSIDASE

Tested at 3% for seven days in vivo with human volunteers, *Pichia anomala* extract stimulates by 38 percent the activity of β-glucocerebrosidase, the enzyme responsible for the synthesis of functional ceramides, compared to a placebo.

After they are secreted, lipid precursors are converted by enzymes into mature, functional lipids and arranged in lamellae.

» Phospholipids form free fatty acids under the action of sphingolipase A2.
» Sphingomyelins and glucosylceramides are converted into ceramides by sphingomyelinase and β-glucocerebrosidase, respectively.

B-glucocerebrosidase is a key enzyme in extracellular lipid metabolism. In the presence of its activator, saponin C, β-glucocerebrosidase hydrolyzes the glucose residues of glucosylceramides, producing mature ceramides in the stratum corneum. B-glucocerebrosidase activity is regulated by acidification of the stratum corneum, with optimal pH at 5.5. Barrier-function disruption increases β-glucocerebrosidase activity and mRNA.

B-glucocerebrosidase deficiency or the absence of its activator can cause major reductions of ceramides, impacting the integrity and function of lipid membranes and leading to a drastic increase of transepidermal water loss.

FUNCTIONAL LIPID CEMENT

Tested at 3% for seven days with human volunteers, *Pichia anomala* extract stimulates the synthesis of non-hydroxylated ceramides by 6.6 percent, cholesterol sulfate by 9.7 percent, and lipids bound to the cornified envelope by 14 percent, compared to a placebo.

Lipid constituents of the extracellular matrix in the cornified layer have unique compositions: ceramides, cholesterol, and free fatty acids. Each lipid family plays an important role in skin hydration and corneocyte cohesion.

Free fatty acids contribute to acidification of the stratum corneum, a process indispensible for enzyme activity, and enable the arrangement of lipid membranes to control water loss.

Cholesterol is the most important sterol of the epidermis, involved in formation of lamellar membranes. Although a minor constituent (2–5 percent), cholesterol sulfate is indispensible to regulate desquamation.

Ceramides in the cornified layer have been categorized into nine subclasses and three distinct families:

» Amide-linked non-hydroxy fatty acids: ceramides NS, NP, and NH (or 2, 3, and 8),
» Amide-linked alpha-hydroxy fatty acids: ceramides AS, AP, and AH (or 5, 6, and 7),
» Ester-linked omega-hydroxy fatty acids or acylceramides: ceramides EOS, EOP, and EOH (or 1, 9, and 4).

Qualitative importance and structural diversity give ceramides their indispensible properties for the organization and function of the permeable barrier. The omega-hydroxyceramides, for example, participate in cohesion of the cornified layer two ways.

First, organization of extracellular lamellar lipids: The structure of the ester-linked molecule makes it act as a molecular rivet, fastening lamellar membranes together, ensuring the stability and cohesion of the whole assembly.

Second, corneocytes have an envelope lined with a residual-lipid membrane that provides cohesion between hydrophilic corneocytes and lipophilic lamellar membranes. These lipids are bound covalently to corneocyte-envelope proteins, and most are omega-hydroxyceramides. The bound lipids give the corneocytes the hydrophobicity necessary to form a rigid envelope. Maturation of the envelope reinforces an insoluble protective structure, which helps maintain the barrier function.

Defective metabolism of epidermal lipids is accompanied by anomalies in the barrier function. Dry skin correlates with reduction of ceramide levels. A decrease in covalently bound ceramides correlates with increase of transepidermal water loss.

Efficacy

EFFECT ON EXOGENOUS LIPIDS

Tested at 0.3% on normal human keratinocytes, mannans of *Pichia anomala* increased by 33 percent the expression of mRNA coding for fatty-acid-transporter protein 3 (FATP-3), thus favoring the mobilization of exogenous lipids into keratinocytes.

The aim of this study was to determine the effect of *P anomala* on expression of mRNA coding for FATP-3 proteins, which are transporters in the mobilization of exogenous lipids into keratinocytes. Tests were conducted on normal human keratinocytes by quantitative polymerase chain reaction (PCR).

Day 0: Normal human keratinocytes were inoculated in six-well plates. Cells were placed in a 37°C incubator containing 5% CO_2.

Day 3: Cells were treated with *P anomala* extract at 0.15% and 0.30% (v/v). Cells were placed in a 37°C incubator containing 5% CO_2.

Turn to Panel 6.

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Moisturizing Treatment for Ultra-Dry Skin

By stimulating the synthesis, transport, secretion, and maturation of epidermal lipids, mannans of Pichia anomala *ensure the function of lipids in the lamellar membranes and corneocyte envelopes.*

Day 9: Cells were recovered. Total RNA was extracted and reverse transcripted. The complementary DNA obtained was analyzed with quantitative PCR. The mRNA of β2-microglobulin, the internal standard, was analyzed in parallel with FATP-3 mRNA.

Fluorescence incorporation (SYBR green) was quantified continuously using a Bio-Rad MyiQ iCycler thermal cycler, with analysis by software. Results are given in Table 1.

Product	Expression of FATP-3
Control	100%
P anomala extract 0.15%	119%
P anomala extract 0.30%	133%

Table 1. Effect of *Pichia anomala* extract on the expression of FATP-3 mRNA

EFFECT ON GLUCOSYLCERAMIDE

Tested at 0.3% on normal human keratinocytes, mannans of *Pichia anomala* led to a 22 percent increase in expression of mRNA coding for glucosylceramide synthase, an enzyme responsible for synthesis of glucosylceramides, precursors of ceramides.

The aim of this study was to determine the effect of *P anomala* extract on expression of mRNA coding for glucosylceramide synthase. This study was conducted on normal human keratinocytes by quantitative polymerase chain reaction (PCR).

Day 0: Normal human keratinocytes were inoculated in six-well plates. Cells were placed in a 37°C incubator containing 5% CO_2.
Day 3: Cells were treated with *P anomala* at 0.15% and 0.30% (v/v). Cells were placed in a 37°C incubator containing 5% CO_2.
Day 4: Cells were recovered. Total RNA was extracted and reverse transcripted. The complementary DNA obtained was analyzed with quantitative PCR. The mRNA of β2-microglobulin, the internal standard, was analyzed in parallel with FATP-3 mRNA.

Fluorescence incorporation (SYBR green) was quantified continuously using a Bio-Rad MyiQ iCycler thermal cycler, with analysis by software. Results are given in Table 2.

Product	Expression of glucosylceramide synthase
Control	100%
P anomala extract 0.15%	108%
P anomala extract 0.30%	122%

Table 2. Effect of *Pichia anomala* extract on expression of mRNA coding for glucosylceramide synthase

EFFECT ON TRANSPORTER PROTEIN

Tested at 0.3% on normal human keratinocytes, mannans of *Pichia anomala* increased by 25 percent the expression of ABCA12, a protein involved in the formation of lamellar bodies and the secretion of their contents.

The aim of this study was to determine the effect of *P anomala* extract on the expression of mRNA coding for protein ABCA12. This study was conducted on normal human keratinocytes by quantitative polymerase chain reaction (PCR).

Day 0: Normal human keratinocytes were inoculated in six-well plates. Cells were placed in a 37°C incubator containing 5% CO_2.
Day 3: Cells were treated with *P anomala* at 0.15% and 0.30% (v/v). Cells were placed in a 37°C incubator containing 5% CO_2.
Day 9: Cells were recovered. Total RNA was extracted and reverse transcripted. The complementary DNA obtained was analyzed with quantitative PCR. The mRNA of β2-microglobulin, the internal standard, was analyzed in parallel with mRNA of ABCA12.

Fluorescence incorporation (SYBR green) was quantified continuously using a Bio-Rad MyiQ iCycler thermal cycler, with analysis by software. Results are given in Table 3.

Product	Expression of ABCA12
Control	100%
P anomala extract 0.15%	107%
P anomala extract 0.30%	125%

Table 3. Effect of *Pichia anomala* extract on the expression of mRNA coding for ABCA12

B-GLUCOCEREBROSIDASE

After seven days of twice-daily application, mannans of *Pichia anomala* formulated at 3% led to a 38 percent increase in the activity of β-glucocerebrosidase, compared to a placebo, after chronic disruption of the lipid barrier by repeated aggression with sodium lauryl sulfate. This effect was observed in 65 percent of volunteers.

The aim of this study was to quantity in vivo with volunteers the effect of *P anomala* extract formulated at 3% in an emulsion on the activity of β-glucocerebrosidase, an enzyme that converts glucosylceramides into ceramides, essential for maintaining optimal barrier function.

The study was conducted with 20 healthy female volunteers 25 to 51 years of age with normal skin on the arms.

Samples of stratum corneum were taken with D-Squame adhesive tape before and after seven days of twice-daily application of *P anomala*. At each measurement, 11 successive samples were taken from the same zone of the skin using an applicator providing constant pressure for five seconds. The last six adhesive samples were stored at –20°C before the assay. The location of sampling sites and their identification at subsequent kinetic points had to be reproducible.

The activity of β-glucocerebrosidase was assayed. The substrate used was 4-methylumbelliferyl-β-D-glucopyranoside. Four-methylumbelliferone, formed by the enzymatic reaction, was determined by fluorometry (excitation 360 nm / emission 450 nm) using a calibration curve in the concentration range of 0–1,500 nM.

Results were expressed as nmol/h/mg protein. Concentration of total proteins in each sample was assayed with the Quanti-Prot BCA kit.

After washing the study zones with 10% sodium lauryl sulfate (SLS), the study extract and placebo were applied to predefined zones of the upper arms for seven days, morning and evening, by massaging gently until penetration. Volunteers were required to wash hands between products.

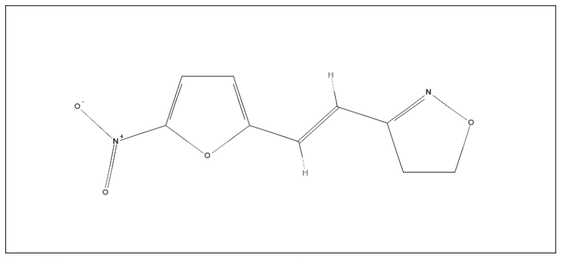

Figure 3. A typical glucosylceramide molecule, one precursor of skin lipids

Prior to the study, volunteers came to the laboratory, signed consent forms, and received information sheets. For seven days before the study, no creams were to be applied to the upper arms.

Day 0
» Volunteers came to the laboratory without applying any product to the arms.
» Measurement zones on the upper arms were determined. Zones were 1) untreated, 2) placebo, and 3) *Pichia anomala* zones.
» Volunteers rested and adapted to surrounding temperature and humidity for 10 minutes.
» Samples were taken from each zone by adhesive stripping.
» Products were distributed.
Day 0 to Day 6
» Study zones were washed twice daily with an irritating SLS-based soap.
» *P anomala* and the placebo were applied twice daily.
Day 7
» Volunteers came to the laboratory without applying any product to the arms.
» Measurement zones on the upper arms were determined.
» Volunteers rested and adapted to surrounding temperature and humidity for 10 minutes.
» Samples were taken from each zone by adhesive stripping.

Twenty volunteers completed the study. The change in enzymatic activity of β-glucocerebrosidase, observed for the study extract and the placebo, was calculated and analyzed statistically using Statgraphics Centurion software. Results are shown in Table 4.

Product	Increase vs. placebo
P anomala extract 3%	+38%

Table 4. Effect of *Pichia anomala* extract formulated at 3% on enzymatic activity

EPIDERMAL LIPIDS MATURATION

After seven days of twice-daily application, mannans of *Pichia anomala* formulated at 3% led to a 14 percent increase in lipids of the cornified cell envelope in the stratum corneum, compared to a placebo, after repeated aggression with sodium lauryl sulfate (SLS). This effect was observed in 60 percent of volunteers.

By stimulating synthesis of corneocyte lipids, *P anomala* extract helped to preserve the architecture of the cell envelope, responsible for maintaining the barrier function.

The aim of this study was to quantify in vivo with volunteers the effect of *P anomala* formulated at 3% in an emulsion on the synthesis of lipids covalently bound to proteins of the cell envelope. These lipids play an important role in cell architecture and the organization of lamellar membranes, maintaining the barrier function.

This effect was observed after seven days of twice-daily application of the study extract and the placebo, following disruption of the barrier function by an irritating SLS detergent to induce disorganization of the lipid structure in the stratum corneum.

The study was conducted with 20 healthy female volunteers 25 to 51 years of age with normal skin on the arms. Samples of stratum corneum were taken with Apli adhesive tape. At each measurement time, two samples were taken using an applicator providing constant pressure for five seconds and were stored at –20°C before labeling.

The location of sampling sites and their identification at subsequent kinetic points had to be reproducible. Measurements were made on symmetrical zones of the upper arms.

After extraction, samples were stained with Nile red, used to determine the hydrophobic nature of corneocytes — the more hydrophobic the sample, the more intense the red fluorescence.

The hydrophobic material stained by Nile red is primarily insoluble components of the corneocyte envelope — lipids covalently bound to proteins — forming the lipid envelope required for homeostasis of the barrier function.

Fluorescence of each sample was observed with an Olympus IX70 microscope equipped with a Nikon DXM1200C digital camera, using Nikon NIS-Elements image-analysis software. Four photos of each sample were taken.

Each image was analyzed using several operations enabling different classes of objects to be distinguished — corneocytes with varying degrees of fluorescence. Average gray level was calculated for each of the four images, and the final result was their mean.

As fluorescent intensity increased, the mean gray level got higher, and the concentration of hydrophobic material, such as lipids, also got higher.

After washing the study zones with a 10% SLS soap, products were applied to predefined zones of the upper arms for seven days, morning and evening, by massaging gently until penetration. Volunteers were required to wash their hands between each product.

Prior to the study, volunteers came to the laboratory, signed consent forms, and received information sheets. For seven days before the study, no creams were applied to the upper arms.

Day 0
» Volunteers came to the laboratory without applying any product to the arms.
» Measurement zones on the upper arms were determined. Zones were 1) untreated, 2) placebo, and 3) *Pichia anomala* zones.
» Volunteers rested and adapted to surrounding temperature and humidity for 10 minutes.
» Samples were taken from each zone by adhesive stripping.
» Products were distributed.
Day 0 to Day 6
» Study zones were washed twice daily with an irritating SLS-based soap.
» *P anomala* and the placebo were applied twice daily.
Day 7
» Volunteers came to the laboratory without applying any product to the arms.
» Measurement zones on the upper arms were determined.
» Volunteers rested and adapted to surrounding temperature and humidity for 10 minutes.
» Samples were taken from each zone by adhesive stripping.

Twenty volunteers completed the study. Results were analyzed using Statgraphics Centurion software. Results are shown in Table 5.

Product	Increase vs. placebo
P anomala extract 3%	+14%

Table 5. Effect of *Pichia anomala* extract formulated at 3% on the mean gray level, representing the concentration of hydrophobic lipids, compared to a placebo

ASSAY OF EPIDERMAL LIPIDS

After seven days of twice-daily application, mannans of *Pichia anomala* formulated at 3% increased the concentration of cholesterol sulfate by 9.7 percent, and the concentration of non-hydroxylated ceramides by 6.6 percent, compared to a placebo. The study showed that 71 percent of volunteers presented an increased cholesterol-sulfate content, and 65 percent had increased non-hydroxylated ceramides.

By favoring synthesis of ceramides and cholesterol sulfate, *P anomala* extract reinforced the barrier function.

The aim of this study was to quantify in vivo with volunteers the effect of *Pichia anomala* formulated at 3% on the synthesis of skin lipids, as observed after seven days of twice-daily application. The study included 18 healthy female volunteers 29 to 61 years of age, selected by a dermatologist on the basis of dry calf skin.

Samples from the stratum corneum were removed by stripping with cyanoacrylate glue before and after the treatment period. The locations of sampling sites and their identification at subsequent kinetic points had to be reproducible. Measurements were taken on symmetrical zones of the calves.

Extracted lipids were separated and identified by high-performance thin-layer chromatography and quantified by densitometry.

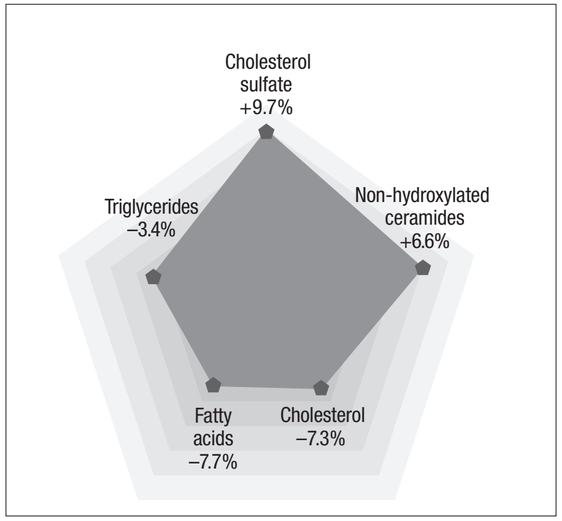

Figure 4. Effect of *Pichia anomala* extract formulated at 3% on synthesis of epidermal lipids, compared to a placebo

Analysis involved quantifying different lipid classes: cholesterol, cholesterol sulfate, cholesterol acetate, hydroxylated ceramides, non-hydroxylated ceramides, fatty acids, and triglycerides.

Results are expressed as a percentage of lipids with respect to protein. Total proteins were assayed with a biochemical method. The quantity of each lipid was used to create a lipid profile.

The study extract and placebo were applied to predefined zones on the calves for seven days, morning and evening, by massaging gently until penetrated. Volunteers were required to wash their hands between each product.

Volunteers came to the laboratory and signed consent forms. The day before the study, no creams were applied to the calves.

Day 0
» Volunteers came to the laboratory without applying any product to the calves.
» Measurement zones on the calves were determined. Zones were 1) untreated, 2) placebo, and 3) *Pichia anomala* zones.
» Samples were taken from each zone with cyanoacrylate glue.
» Products were distributed.
Day 0 to Day 6: *P anomala* and the placebo were applied twice daily.
Day 7
» Volunteers came to the laboratory without applying any product to the calves.
» Measurement zones on the calves were determined.
» Samples were taken from each zone with cyanoacrylate glue.

Eighteen volunteers participated and 17 completed the study. Data were analyzed by a predetermined formula. Results for cholesterol sulfate, non-hydroxylated ceramides, cholesterol, fatty acids, and triglycerides are shown in Table 6 and Figure 4.

Lipid	Variation vs. placebo
Cholesterol sulfate	+9.7%
Non-hydroxylated ceramides	+6.6%
Cholesterol	–7.3%
Fatty acids	–7.7%
Triglycerides	–3.4%

Table 6. Effect of *Pichia anomala* extract at 3% on synthesis of epidermal lipids, compared to a placebo

BARRIER EFFECT

After 14 days of twice-daily application, mannans of *Pichia anomala* formulated at 3% reduced transepidermal water loss by 14 percent, compared to a placebo, after repeated aggression by sodium lauryl sulfate. This effect was observed in 75 percent of the volunteers.

P anomala extract limits derangement in the lipid structure of the stratum corneum caused by repeated application of sodium lauryl sulfate, thereby favoring optimal barrier function.

The aim of this study was to quantify in vivo with volunteers the effect of *P anomala* formulated at 3% in an emulsion on transepidermal water loss. The effect was observed after seven and 14 days of twice-daily application following chronic artificial disruption of the barrier function by the detergent sodium lauryl sulfate.

The study was conducted with 20 healthy female volunteers 25 to 51 years of age with normal skin on the arms.

Measurements were taken with a Tewameter TM 210 equipped with a probe to measure water-vapor exchange between skin and surrounding air, a reflection of the barrier function of the stratum corneum.

The location of sampling sites and their identification at subsequent kinetic points had to be reproducible. Measurements were taken on symmetrical zones of the upper arms.

After washing the study zones with 10% sodium lauryl sulfate, the study extract and placebo were applied to predefined zones on the upper arms for 14 days, morning and evening, by massaging gently until penetration. Volunteers were required to wash their hands between each product.

Volunteers came to the laboratory, received an information sheet, and signed a consent form. For seven days before the study, no creams were applied to the arms.

Day 0
» Volunteers came to the laboratory without applying any product to the arms.
» Measurement zones on the upper arms were determined. Zones were 1) untreated, 2) placebo, and 3) *Pichia anomala* zones.
» Volunteers rested and adapted to surrounding temperature and humidity for 10 minutes.
» Tewameter measurements were taken from each zone.
» Products were distributed.
Day 0 to Day 6
» Volunteers washed the study areas twice daily with an irritating sodium-lauryl-sulfate soap.
» *P anomala* extract and the placebo were applied twice daily.
Day 7
» Volunteers came to the laboratory without applying any product to the arms.
» Measurement zones on the upper arms were determined.
» Volunteers rested and adapted to surrounding temperature and humidity for 10 minutes.
» Tewameter measurements were taken from each zone.
Day 7 to Day 13
» Volunteers washed the study areas twice daily with an irritating sodium-lauryl-sulfate soap.
» *P anomala* and the placebo were applied twice daily.
Day 14
» Volunteers came to the laboratory without applying any product to the arms.
» Measurement zones on the upper arms were determined.
» Volunteers rested and adapted to surrounding temperature and humidity for 10 minutes.
» Tewameter measurements were taken from each zone.

Twenty volunteers completed the study. Results for transepidermal water loss with the extract and the placebo were analyzed by a predetermined formula and are shown in Table 7.

Day	TEWL reduction of *P anomala* vs. placebo
Day 7	–6%
Day 14	–14%

Table 7. Effect of *Pichia anomala* extract at 3% on transepidermal water loss over time, compared to a placebo

SAFETY TESTS

NON-IRRITANT, NON-TOXIC

Tests	Results
» Evaluation of skin safety of a cosmetic product after a single application of an occlusive bandage for 48 hours	non-irritant
» Evaluation of sensitizing potential in adult volunteers with normal skin	non-sensitizing
» Mutagenicity	non-mutagenic
» Phototoxicity	non-phototoxic
» Evaluation of irritant potential	non-cytotoxic

DIRECTIONS FOR USE

Twice per day, after washing with a high-quality cleanser, gently pat dry and apply a pea-size amount on the entire face, avoiding the eye area. The morning application should be followed with a sunscreen.

INGREDIENTS

Primary ingredient: *Pichia anomala* extract
Inactive ingredients: deionized water, gum tragacanth, xanthan gum, glycerol (vegetable origin), *Prunus amygdalus dulcis* oil, *Anthemis nobilis* flower extract, vegetable oil, arachidyl alcohol, behenyl alcohol, arachidylglucoside, cetyl ester, C20-C22 alkyl phosphate, phenoxyethanol

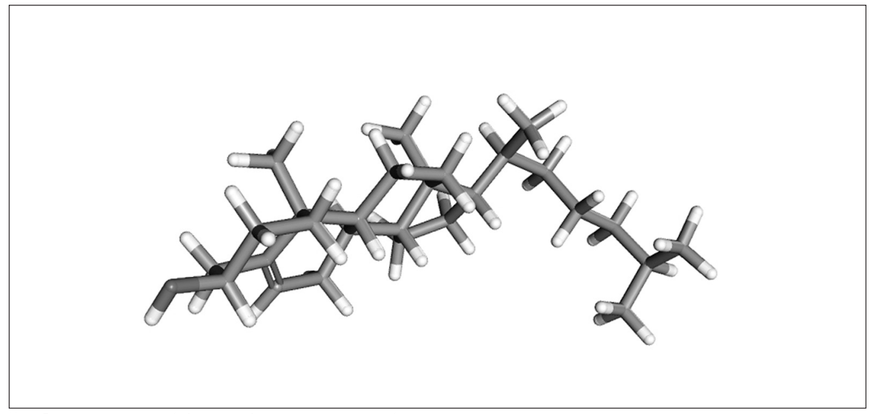

Figure 5. Three-dimensional view of a typical cholesterol molecule, one precursor of skin lipids