New active ingredient proven to shrink pigmented spots

A 100% natural ingredient that is proven to address a major sign of skin ageing

CORINNE REYMERMIER*, VINCENT BARDEY, CATHERINE BONNAUD-ROSAYE, VALÉRIE ANDRÉ, CAROLE BOURY, CÉCILE KALEM
*Corresponding author
BASF Beauty Care Solutions, France

Abstract

Pigmented spots are widely perceived as a sign of skin ageing. These spots occur when the enzyme tyrosinase, which is mainly involved in melanin synthesis, is overproduced because of ageing or overexposure to UV light and pollution. Researchers recently conducted in vitro tests to explore the potential of a new active ingredient extracted from the Lansium domesticum plant to reduce tyrosinase and melanin levels in the skin through a previously unknown epigenetic pathway. In vivo testing then confirmed that this 100-percent natural ingredient decreases the surface area of pigmented spots and reduces the total quantity of melanin. This opens up opportunities for cosmetics manufacturers to meet demand for anti-ageing skincare products that address pigmented spots.

Keywords

- Pigmented spots
- Skin care
- Tyrosinase
- Melanin
- Epigenetic mechanism
- Lansium domesticum
- Peer Reviewed

ADDRESSING A COMMON SIGN OF SKIN AGEING

Signs of ageing skin include wrinkles, crow's feet and pigmented spots. Many consumers use moisturisers to smooth out the first two signs – and a new natural ingredient has now demonstrated efficacy in addressing the third. *In vitro* and *in vivo* testing has shown that an extract of the *Lansium domesticum* plant shrinks and lightens pigment spots on the skin, creating a more homogenous skin tone that is associated with youthful appearance. This 100% natural ingredient with the INCI name Maltodextrin (and) Lansium Domesticum Leaf Extract leverages a new epigenetic mechanism to deal with pigment spots at their root cause rather than just on the surface.

Pigmented spots on the face, hands and décolleté area are among the most common visible signs of photoaging. Studies have demonstrated that the formation of these spots is accelerated by exposure to UV light and traffic-related air pollution. An observational study in Japan showed that women from Kagoshima have a significantly higher degree of facial hyperpigmentation than women from Akita, where there is less UV radiation (1). A study in China showed an increase of 20 µg/m^3 in coarse particular matter (PM10) was associated with 16% more pigment spots on the cheeks (2, 3). These studies of Asian skin types are particularly relevant because pigmented-spot intensity has been shown to be 20-times more prevalent in Chinese women than French Caucasian women, while spots appeared earlier on Japanese women than on French Caucasian women (4).

A NEW EPIGENETIC MECHANISM

The process of skin pigmentation involves the production of melanin by melanocyte cells, which then distribute melanin throughout the epidermis. As people get older, UV exposure and other environmental factors can cause an overproduction of tyrosinase, a key enzyme involved in melanin synthesis. This causes pigment spots (5). For this reason, controlling tyrosinase production can reduce the creation of pigmented spots. Recent scientific research has explored how short nucleic acid molecules known as microRNA (miRNA) can be used to regulate melanin production. The research discovered that UV radiation inhibits the miR-490-3p molecule, which controls tyrosinase production in melanocytes (Figure 1). As a result, in vitro and in vivo testing were conducted to explore the potential for Maltodextrin (and) Lansium Domesticum Leaf Extract to stimulate miR-490-3p and provide an epigenetic solution to shrink and lighten pigmented spots on the skin.

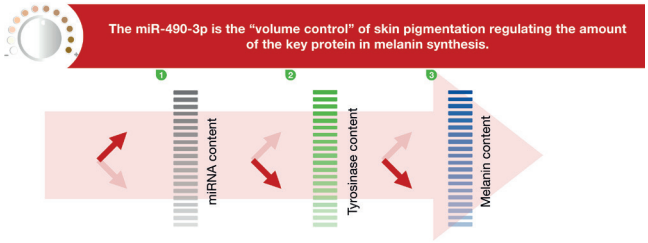

Figure 1. Treatment with Lansium Domesticum leaf extract increases the level of miR-390-3p, which contributes to decreasing the level of Tyrosinase. This ultimately decreases melanin content.

Maltodextrin (and) *Lansium Domesticum* Leaf Extract is a 100% natural active ingredient that comes from the leaves of the langsat tree (Figure 2). The extract obtained is standardised in plant dry matter and available as a preservative-free powder. Its typical phytochemical composition is represented in Figure 3. In addition to 2-4% polyphenols (expressed in equivalent of tannic acid), the plant extract contains dukunolides which have been identified by mass spectrometry.

Figure 2. The Lansium domesticum plant is valued across South East Asia because its fruit, peel, bark, seeds and leaves display antioxidant properties.

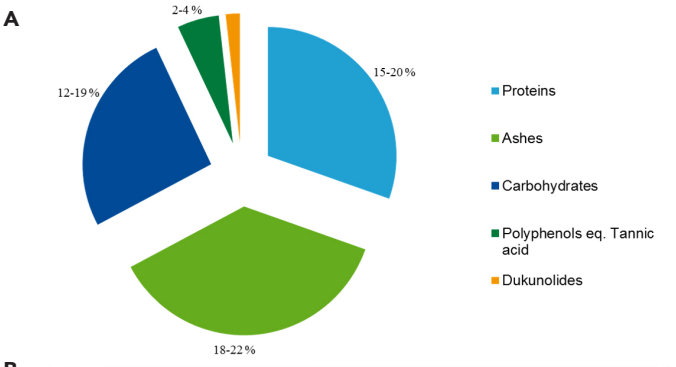

Figure 3. Phytochemical data: A – Overview of the typical phytochemical composition of the plant extract matter (based on three representative batches). B – Mass spectrometry of dukunolides present in the leaves of Lansium domesticum. C – Molecular structure of dukunolides (dukunolides' presence was validated by HPLC-MS analysis. No quantification due to lack of standards).

RESULTS AND DISCUSSION

In vitro trials have demonstrated that this active ingredient supports the improvement of the regulation of melanin synthesis through an epigenetic pathway with three key areas of efficacy – it increases the level of miR-490-3p, while decreasing the level of tyrosinase and melanin (Figure 1).
The trial used a culture of Asian human foreskin melanocytes that were derived from surgery. The ingredient was applied at concentrations of 0.034% and 0.07%, and the miR-490-3p expression was tested after 24 hours. At a concentration of 0.07%, the active ingredient increased the level of miR-490-3p by a factor of 1.4. Results at the lower dose were not significant. (Figure 4). Additional studies demonstrated that the extract at 0.034% decreases the level of tyrosinase in a melanocyte culture by 33% after 72 hours of treatment (*Figure 5*). And it was also shown to decrease the level of melanin in a co-culture of human melanocytes and keratinocytes by 34% after four days of treatment with a formula containing 0.07% (*Figure 6*).

In vivo trials subsequently confirmed the positive impact of this active ingredient by decreasing the surface area of pigment spots and reducing the total quantity of melanin. This trial involved 53 Chinese women aged 18-60 who live in Guangzhou, a sunny and polluted area of China. All of these women exhibited well-defined pigment spots on both halves of the face before the double-blind, randomised split-face study began. The active ingredient was applied in a cream formula with a concentration of 0.3% and compared to a placebo and a benchmark product containing Ascorbyl Glucoside at a 2% concentration. After twice-daily application of the creams for 56 days, the surface area of the pigment spots and the total quantity of melanin were measured using image analysis (Figure 7). The active ingredient achieved a decrease of 23% in both parameters and was significant versus placebo, while the benchmark product did not show any effect (Figure 8).

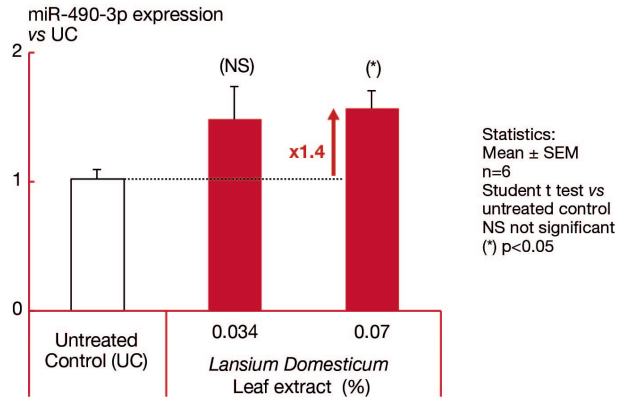

Figure 4. Quantification of the expression of miR-490-3p by Q-RT-PCR in human melanocytes after 24 hours with or without treatment with the active ingredient.

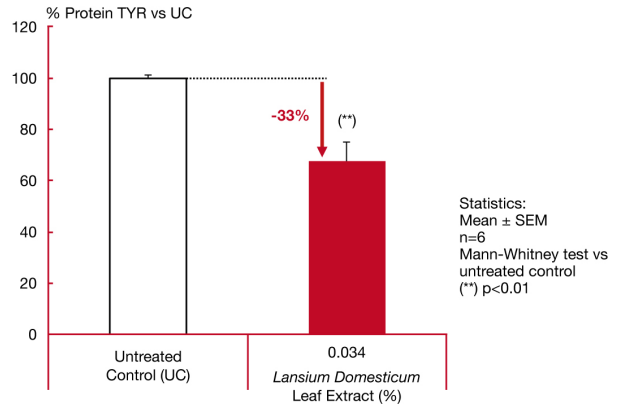

Figure 5. Quantification of TYR protein synthesis in human melanocytes after 72 hours with or without treatment with the active ingredient.

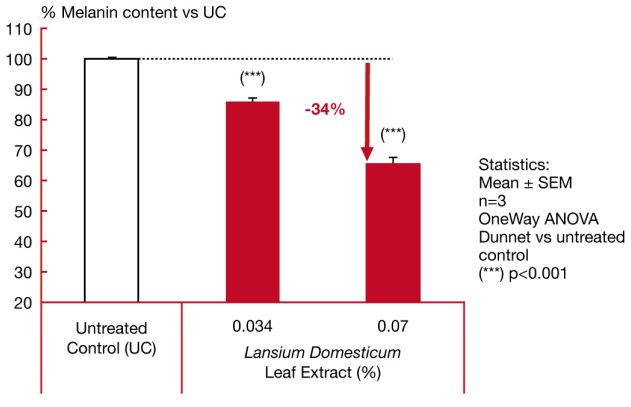

Figure 6. Quantification of melanin synthesis in melanocytes and keratinocyte co-culture, after 4 days with or without treatment with the active ingredient.

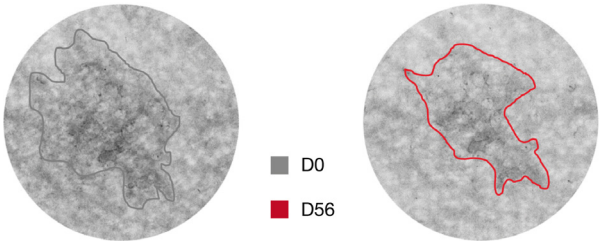

Figure 7. Illustrative pictures of a defined pigmented spot, analysed in grey level, before and after treatment with the new active ingredient at 0.3%.

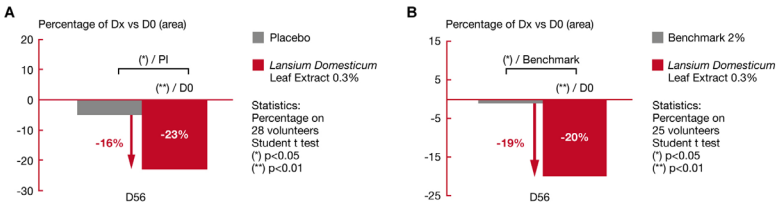

Figure 8. Percentage of pigmented spot's melanin content decrease after 56 days of application on the face vs baseline (D0) and compared to placebo (A) and Benchmark (AA2G at 2%) (B).

IN VITRO MATERIALS AND METHOD FOR MEASURING EXPRESSION OF MIR-490-3P IN MELANOCYTES

The *in vitro* tests to demonstrate miR-490-3p expression in melanocytes were carried out using Asian normal human foreskin melanocytes derived from surgery.
The cells were treated with or without the active ingredient at a concentration of 0.034% and 0.07%, for 24-hours. Then, total RNAs were extracted for quantitative real-time polymerase chain reaction (Q-RT-PCR) analysis. Assessment of the expression level of miR-490-3p was performed using the Taqman microRNA assay method One-step RT-PCR kits (TaqMan MicroRNA Reverse Transcription and TaqMan Fast Advanced Master Mix) with miR-490-3p Exiqon primer. The values from each experiment were measured using a quantitative thermocycler. The results were normalised using RNU6B as a housekeeping gene and all of the assessments were performed in triplicate on each biological replicate. Results were normalised to the untreated condition (UC) and expressed as the mean (fold change) ± standard error of mean (SEM) from triplicate assay. The statistical analysis was carried out using Student t test and the significance threshold was fixed at 0.05 for the p-value (*Figure 4*).

IN VITRO MATERIALS AND METHOD FOR MEASURING TYROSINASE MODULATION

The *in vitro* tests to demonstrate tyrosinase modulation were also carried out using Asian normal foreskin melanocytes derived from surgery. In this experiment, cells were treated with or without the active ingredient at a concentration of 0.034% for three days. After treatment, the cells were harvested and lysed. Protein concentration was determined by bicinchroninic acid (BCA) assay and the samples were adjusted at 0.5 mg/ml. Targeted Tyrosinase protein was quantified using a capillary electrophoresis-based protein analysis system (Sally Sue: ProteinSimple, San Jose, California, USA) with primary antibody against tyrosinase (TYR) and a horseradish peroxidase-conjugated secondary antibody detected by chemiluminescent substrate. The resulting chemiluminescent signal was detected and quantified by the Compass Software version 2.7.1 (ProteinSimple, San Jose, California, USA). The results were normalised to the UC and expressed as the mean (%) ± SEM from two experiments in triplicate assay. The statistical analysis was carried out using the Mann-Whitney test and the significant threshold was fixed at 0.05 for the p-value.

IN VITRO MATERIALS AND METHOD FOR MEASURING MELANIN SYNTHESIS

The *in vitro* test to demonstrate melanin synthesis was carried out using a co-culture of Asian normal human foreskin melanocytes derived from surgery with keratinocytes (cell line HaCat) (6). Cells were mixed and cultured (50:50) for 6-7 days at 37°C and CO 5% before treatments with the active ingredient at 0.034% and 0.07% concentration for four days. The level of intracellular melanin was measured by recording the optical density at 475 nm of cells' homogenate.
The results were expressed as the mean values (%) ± SEM from triplicate assay. The statistical analysis was carried out using the OneWay ANOVA test followed by the Dunnet test, and the significance was fixed at 0.05 for the p-value.

IN VIVO MATERIALS AND METHOD FOR MEASURING PIGMENTED SPOT AREA AND MELANIN CONTENT

The *in vivo* study to analyse pigmented spot area and melanin content was carried out as a double-blind, randomised, split-face study under dermatological control. The test was performed in Guangzhou, a sunny and polluted region of China. Two independent groups of female volunteers, aged 18 to 60, were involved. All participants, as a specific inclusion criterion, presented pigmented spots on each half of the face belonging to the phototypes III and IV on the Fitzpatrick grading. The active ingredient at a concentration of 0.3% was evaluated, as were Ascorbyl Glucoside (AA2G) at a concentration of 2%, and a placebo cream. Each volunteer within each of the two groups tested two different products, applied to one half of a clean face twice each day under normal conditions of use. Checkpoints were performed at Day 0 (baseline) and Day 56. High-resolution digital photographs of the face were taken in cross-polarised light (SIAscope). One pigmented spot of 3 to 7 mm in diameter was selected on each half of the face according to colour intensity and contours, and the area of this pigmented spot and the total quantity of melanin were measured using image analysis (Spectroscan – Orion Concept, France).

The results were expressed as the mean percentage of change compared to the baseline measurement. The statistical analysis of the evolution of the parameters in function of products was done after the verification of the normality of distribution using the Shapiro-West test. Afterwards, the statistical analysis of the evolution of the studied parameters for each product was performed with the Student t test, when the normality of distribution was confirmed, or with a non-parametric test (Mann-Whitney Rank Test).

OPENING UP OPPORTUNITIES FOR ANTI-AGEING SKIN PRODUCTS

The *in vivo* and *in vitro* tests on Maltodextrin (and) *Lansium Domesticum* Leaf Extract clearly show that this active ingredient is able to increase the level of miR-490-3p and decrease the levels of tyrosinase protein, which in turn decreases the area of pigmented spots and reduces the level of melanin. Overall, this demonstrates that this new epigenetic mechanism is effective in making pigmented spots on the skin smaller and lighter.
This effect increases the homogeneity of the skin, which is often associated with a youthful appearance. For this reason, this new active ingredient provides cosmetics manufacturers with an innovative new way of creating anti-ageing skin products that offer consumers a proven mechanism for addressing one of the most common visible signs of photoaging – and achieving younger-looking skin.

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ABOUT THE AUTHOR

Corinne Reymermier, R&D Project Leader, Graduated in 1996 from the UCBL1 University in Lyon (France), Master 2 research in biological and medical engineering, she joined BASF in 2006 as a R&D project manager. Since 2014, she works on epigenetic concepts research for new innovative cosmetic solutions through the epigenetic platform management to develop knowledge and discover new solutions for the market needs.