

COSMETICS INSPIRED BY INTEGRATIVE MEDICINE

Could **complementary natural bioactives** from plants be used to produce more advanced skin care?

Joint interview with Pr. Jan Frank from Institute of Biological Chemistry and Nutrition at the University of Hohenheim in Stuttgart, Germany, Jocelyne Franchi, Scientific Coordinator for Dior Science, France and Laure Pasquier, Researcher at LVMH Recherche, France.

We are hearing a lot about Integrative Medicine these days as a more holistic approach to healthcare. The idea here is that complementary treatments, such as phytotherapy and nutrition, when used in conjunction with conventional therapies, should lead to better overall results.

Plants naturally contain a plethora of chemical molecules, some of which may have nutritive properties or antioxidant, antibacterial, antifungal or anti-inflammatory activity. "In its simplest form, Integrative Medicine is when patients are given high doses of plant compounds - curcumin and resveratrol are two better-known examples here - in

addition to standard medical treatments," explains Jan Frank of the Institute of Biological Chemistry and Nutrition at the University of Hohenheim in Stuttgart, Germany. "Curcumin (which is the yellow dye from the rhizome of *Curcuma longa*, or turmeric) is being studied for its potential as an adjuvant therapy, for instance, because many recent cell culture and animal studies have shown that it can act as an anti-inflammatory."

SYNERGISTIC TREATMENTS LEAD TO BETTER EFFICACY

"Together with Mohamed Khayyal's team at the University of Cairo, Egypt, we recently found that curcumin in a certain (micellar) form is more potent than diclofenac in reducing concentrations of C-reactive protein (a marker of systemic inflammation) in an animal model of arthritis," he says. "When combined with micellar boswellic acids, derived from the *Boswellia* plant, micellar curcumin reduces

the concentrations of C-reactive proteins even further in arthritic rats.

"Another study has shown that a two-month treatment with $\omega 3$ fatty acids plus curcumin decreases the frequency of migraine attacks in a trial with 74 patients. It does this by reducing the concentration of the inflammatory cytokine TNF α in blood serum compared to a placebo or to treatment with either of the bioactive molecules alone."

Using two or more bioactive molecules in such a complementary, synergistic way allows the compounds to act on different molecular targets in the body at the same time. "In nutritional medicine, for example, one compound may reduce the absorption of cholesterol in the intestine while another the synthesis of new cholesterol in the liver," explains Frank. "Again, if both compounds are allowed to act together, they decrease cholesterol levels to a greater extent than either one of them alone."

BIOACTIVES IN COSMETICS

“The same principle could be applied to the world of cosmetics, with different bioactives targeting different aspects of skin ageing, for example,” he says. “One substance might help protect the skin from the damaging effects of ultraviolet radiation while another help regenerate skin cells. A third active could act on chronic inflammations. If all these compounds are combined in a single treatment, they should produce better results than any one of them by itself.”

Today, such bioactive molecules can be readily extracted and isolated from plants, and then characterized and authenticated using a variety of macroscopic and microscopic techniques, metabolite profiling and metabolomics analysis. Indeed, techniques like high performance liquid chromatography (HPLC) combined with electrospray ionization and quadrupole-time-of-flight mass spectrometry in conjunction with principal component analysis (PCA) have proved themselves to be very useful here. Researchers at LVMH Recherche for Dior in France have been using some of these technologies and many others for studying different micronutrients or bioactive molecules in a particular hybrid of rose, the “Rose de Granville”. They have also been looking at how these bioactives might be beneficial for skin thanks to their anti-inflammatory action and nutritive properties.

THE ROSE DE GRANVILLE

Humans have been cultivating roses since Antiquity using cross-pollination techniques to produce hybrid species that are more attractive or which are better for making perfumes because they contain more essential oils. Roses are also known to have astringent and anti-ageing properties since they contain molecules that can improve skin tonicity.

The recently produced Rose de Granville (RDG) variety took over 10 years of stringent selection by rose breeders and was made at the request of the cosmetics group Parfums Christian Dior – who has been using it in some of its skin care products for a few years now.

“The RDG has many exceptional properties,” explains Laure Pasquier of

LVMH Recherche in Saint-Jean-de-Braye in France, who has been working on analyzing this flower. “For one, it is twice as robust as an ordinary rose and extremely resistant. It also contains a multitude of nutritive and anti-inflammatory phyto-compounds.”

To better understand this rose and find out which molecules might be the best for ultimately incorporating into a skin care product, Pasquier and her team have now studied its phytochemistry in detail for the first time and have analyzed its molecular composition and the compounds (especially non-volatile ones) it contains. The researchers did this by analyzing three extracts obtained not only from the flower but also from its fruit (rosehip). The different parts of the plant have different functions and thus contain very different chemical molecules that nonetheless complement each other perfectly.

A different extraction method was carefully developed for each of the three extracts to bring out the very best properties of each (see “Extraction techniques”).

THE EFFECTS OF THE MOLECULES ON SKIN

In their latest studies, Dior Researchers were looking in particular for improved cosmetic treatments for the area around the eye, which is quite different to that of the face in that it is much more fragile. For one, it is thinner (to make blinking easier), but it also contains less collagen, elastin and fatty tissue, which means that is structurally less dense. The fact that this zone also contains as many as 22 muscles, 14 of which are activated every 10 seconds or so to make sure the eye remains moist also deteriorates the skin’s elastic structure over time. It contains more blood vessels too, which makes dark circles more apparent for some people, especially when they are tired.

Phytotherapy using molecules obtained from plants like the RDG might come into its own here. Indeed, the Dior researchers have already shown that an extract from the RDG rosehip stimulates the production of ATP in cultured human keratinocytes by 21% since it appears to impact on more than 60 biological targets in skin cells.

“The main micronutrients in this part of the plant are potassium and calcium,”

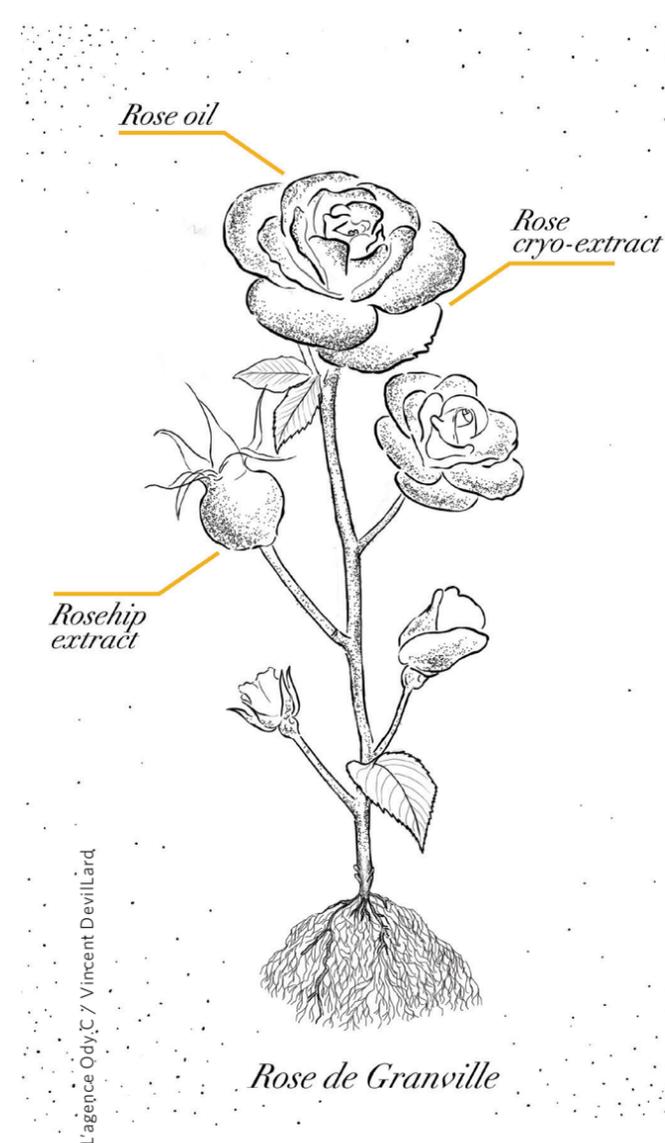
explains Jocelyne Franchi, science coordinator for Dior Science. “These are also present in flower cryo-extracts but in a smaller amount. Calcium is important for the epidermis since it helps reinforce its barrier function. Indeed, we found that the fruit extract stimulates the genetic expression of biomarkers involved in epidermis cohesion and differentiation.

COMBINING EXTRACTS FROM ALL THE DIFFERENT PARTS OF A PLANT, EACH OF WHICH HAVE VERY DIFFERENT BUT COMPLEMENTARY BIOACTIVITY, CAN THUS MAKE FOR AN ADVANCED TREATMENT.

“The extract also stimulates genetic expression of the proteins collagen I, III, V and elastin in the extracellular matrix,” she adds, “as well as the enzymes involved in the formation of the protein fibers LOX (Lysyl oxidase) and PECOL (Procollagen C-endopeptidase enhancer). It inhibits the activity of enzymes that degrade collagen and elastin (MMP3) too.”

Extracts from the rose flower itself contain many essential fatty acids such as $\omega 3$ and $\omega 6$ as well as micronutrients like α -tocopherol (Vitamin E) and β -sitosterol (which is a phytosterol). The cryo-extract of rose, for its part, contains as many as 20 micronutrients, including Vitamin B3, zinc (an oligo-element) and magnesium.

“Combining extracts from all the different parts of a plant (in this case, the flower and rosehip), each of which have very different but complementary bioactivity, can thus make for an advanced skincare treatment,” says Franchi. “What is more, these different extracts also address the special needs of the target zone (the skin around the eye in our study) and show improved efficacy compared to simply one of the extracts alone. This zone is reinforced thanks to the micronutrients in two of the extracts and the anti-inflammatories in another (see Focus).”



EXTRACTION TECHNIQUES

The researchers employed two techniques for extracting the different types of molecules present in the flower. The first is cryo-extraction, which extracts hydrophilic molecules at low temperatures so as to preserve their biochemical properties as much as possible. The second, dynamic enflourage, is an oil-based extraction method in which the plant is zapped with microwaves (at 1000 W power for 30 or 40 seconds) and ultrasound. The goal here to extract the greatest number of molecules, even those that are normally inaccessible, explains Pasquier.

“We adapt our extraction method to specific parts of the plant,” she says. “For example, for the rosehip (the fruit of the rose plant, which begins to form in late spring/early summer), we need to heat the plant but not too much, since it is very sensitive to elevated temperatures. We have thus had to adjust the temperature at each stage of the process so as not to destroy the precious bioactive ingredients it contains. We called it thermo-extraction.

“Our extraction techniques are ‘kind’ to the plant as well as being environmentally friendly and safe for the researchers and

technicians carrying out the experiments,” she adds.

Once they had extracted the different molecules from the flower, Pasquier and colleagues then employed advanced gas and liquid chromatography techniques coupled with a Diode Array Detector, an Evaporative Light Scattering Detector and high-resolution mass spectrometry to analyze the molecular composition of the extracts. They also used metabolomics (in collaboration with researchers at INRA in Versailles, France) and Inductively Coupled Mass Spectrometry (ICP-MS) to analyze certain micronutrients present in the plant (that it had very efficiently absorbed from the soil it was grown in – another one of its good points).

“We used an innovative analytical strategy to compare the molecular compositions in different parts of the rose – namely, wood, bud, flower and leaf,” explains Pasquier. We started by obtaining general information on the major molecular families (which include polyphenols, triterpenes, fatty acids, glycolipids and triglycerides) and then progressively refined the level of analysis to obtain the specific molecular fingerprints of the extracts.”

FOCUS: ANTI-INFLAMMATORY ACTION

Inflammation, like that from sunburn for example, can be seen on the skin straight away, but invisible inflammation is occurring all the time, because of exposure to UV rays (even in winter), to free radicals (external and internal) and a host of other factors. The RDG rosehip extract appears to inhibit the genetic expression of NFKB1, which is a transcription factor involved in inflammation, as well as that of protein pro-inflammatory markers (such as cytokines et genes involved in eicosanoid signaling, which are pro-inflammatory lipid markers for the synthesis of PGE2 or prostaglandins).

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