# Stabilised *Potentilla* polyphenols fight skin ageing

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Over time the ageing process changes skin structure. Damage caused by free radicals prevents normal cellular function and, among other things, the different skin layers become atrophied. The cutaneous relief quality is degraded, skin becomes rough, loses its firmness and wrinkles begin to form. These ageing signs, that appear on the skin surface, have to be reversed from the depth and not just visibly improved.

Consumers become aware that skin ageing is an unavoidable phenomenon: it happens to each of us, all the time and everywhere, no matter our lifestyle. However, we embrace a new age of ageing: self-confidence is no longer just about looking younger – because growing older is better and better accepted – but also to look good for yourself. The search for new action mechanisms is still required to decrease side effects and to improve appearance together with skin texture. Some women are willing to use aesthetic treatment in order to improve their appearance and some others prefer a more natural way: using efficient molecules extracted from plants.

## Skin ageing: a natural process

Skin is made up of three layers: the epidermis, the external layer in contact with the outside which has a protection role, the dermis which represents skin structure, and the hypodermis which is highly vascularised and contains fatty tissues.

Cutaneous ageing is a complex biological process which results from a combination of endogenous or intrinsic (genetics, cellular metabolism, hormone) and exogenous or extrinsic (light exposure, pollution, chemicals and so on) factors. These factors induce structural and physiological alterations. On one hand, the epidermis thickness evolves in consequence of the dermo epidermal junction levelling. On the other hand, the dermis becomes atrophied due to the fibroblast decrease, leading to collagen and elastin fibre depletion. The activity and number of melanocytes decline, leaving cells without protection from UV rays and therefore also from free radicals. Damage caused by free radicals slows down cellular renewal and skin is unable to repair itself.[1,2] Skin loses its firmness and suppleness, its surface becomes rough.

In particular, ageing is the result of functional and structural modifications which occur in-depth, mainly in the dermis and epidermis, and lead to visible signs. On its surface, skin presents irregularities defined as cutaneous relief. This one reflects skin texture quality and the appearance of ageing signs according to two indicators: micro-relief and wrinkles. The micro-relief is characterised by a certain number of depressions which cross each other. These depressions are formed by the association of furrows and folds, and by corneocyte projection. Specific to each body area, the micro-relief changes over the years. Thus, the formed cutaneous lines are directed in all directions when the subject is young – isotropic state – and are aligned when the subject becomes older – anisotropic state.[3] The small furrows disappear gradually with the loss of skin elasticity to cause a state where only deep furrows remain. Wrinkles are the collation of these deep furrows. Visible to the naked eye, they are caused by several phenomena: the atrophy of dermis and epidermis, the loss of skin elasticity and the reorganisation of the three-dimensional network of collagen fibres.

Natural compounds derived from plants, polyphenols are well known to fight against skin ageing. Their great antioxidant and scavenging abilities make them an interested target for research.

*Potentilla.*

## Stabilised polyphenol

Polyphenols are the most powerful antioxidants in the plant world. Very widespread compounds in nature, humans do not have the capacity to synthesise them. They must be brought by food or topical application. From a large class of active substances, they are characterised by a common chemical structure made up by at least one aromatic cycle with one or more hydroxyl functions (-OH). Their properties, such as the intensity of antiradical activity, depend on many factors, for example the number of hydroxyl groups bound to the aromatic cycle or the

molecular size. The more its structure is complex, meaning composed of a large number of aromatic cycles, the more the molecule has strong antioxidant activity. That is the reason why OPC (procyanidolic oligomer) molecules are the most powerful ones. These molecules are composed of flavan-3-ol monomer succession - also called catechin - and are polymerised structures. Unfortunately, they are known as being unstable especially when highly purified. Certain factors, for example light, air and even acid or basic pH, can cause their degradation – such as the oxidation of free phenolic functions and decrease the effectiveness of these molecules.

From this assessment, a method for stabilising polyphenols has been developed and patented by Berkem: an esterification process called Phytovector® technology (Fig 1). This esterification technique consists of masking the free phenolic functions with ester functions, which stabilises polyphenols. As a result, the polyphenols acquire lipophilic characteristics, which are essential to transcutaneous passage and for a good bioavailability.[4,5] The active form of stabilised polyphenols is released inside the epidermis due to the action of esterase enzymes naturally found on skin surface.[6,7]

Several plant extracts have been developed and tested for their high polyphenol content. *Potentilla*, a traditional medicine and flowering plant, showed a great potential.

## Potentilla, a tremendous polyphenol source

Potentilla, *Potentilla erecta* (L.) Raeusch, is a herbaceous plant native to Europe and Western Asia. In Latin, its name means 'little powerful one' in reference to its medicinal qualities such as its antiseptic and soothing properties. *Potentilla*, part of the Rosaceae family, grows from 5 cm to 50 cm in height. The leaves, attached to short petiols to the rising or trailing stalks or directly to the main stem, are pinnate and palmate. Leaves may create rosettes of basal leaves. The small yellow flowers of this specific species have four notched petals.[8] The receptacle is slightly raised and covered with numerous free styles. The part used is the thick, brown and tuberous rhizome. This rapidly turns blood red in colour when freshly cut, due to its high phenolic compound content. In order to study and evaluate its efficiency in the cosmetic field, a new potentilla rhizome extract with high polyphenol content - more than 95% - has been developed. Derived from controlled sourcing, the rhizomes used are harvested in the wild in Europe. The active ingredient derived is called Berkemyol potentilla (INCI name: Palmitoyl Potentilla Erecta Root Extract).

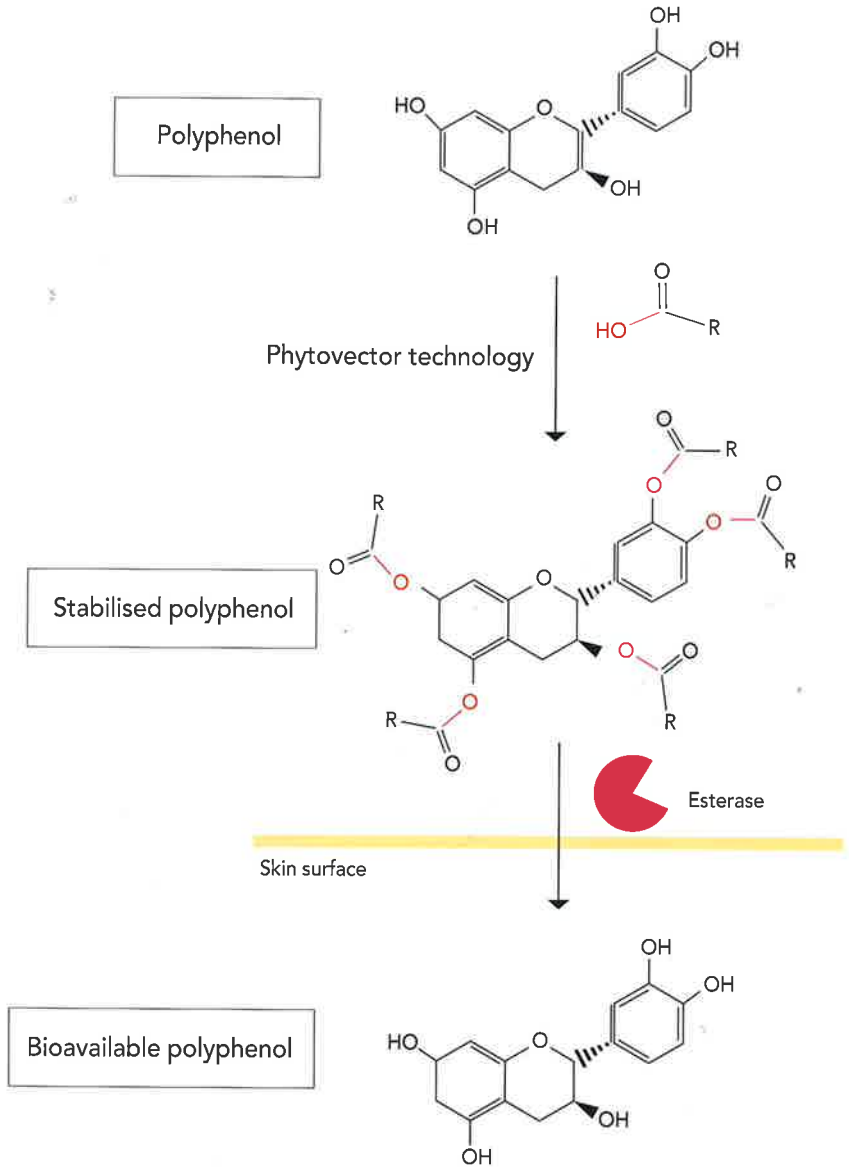


Figure 1: Phytovector® technology.

## Epidermal and dermal structure improvement: *ex vivo* studies

### Effect on oxidative stress

Induced by UV rays, pollution or stress, free radicals cause oxidative damage in cellular DNA, and in the skin's proteins and lipids, which undergo changes and can no longer fulfill their function. The accumulation of oxidised compounds leads to cellular dysfunction and accelerates the cutaneous ageing process.

The study of the anti-radical activity was conducted against free radicals induced by UV irradiation on skin explants. Indeed, under the effect of UV radiation, free radicals are produced on the skin surface and lead to the degradation of cellular components such as membrane lipids. These lipids are transformed into hydroperoxide by-products, in particular malondialdehyde (MDA), one of the main markers of these degradation products and a good indicator of membrane lipid peroxidation. The determination of MDA was carried out on skin explants of a 40-year-old woman donor during four days of treatment. On the fifth day, skin explants are irradiated by UV rays two hours after treatment. Irradiation is carried out with a UV simulator in UVA at the dose of 18 $J.cm^{-2}$ and in UVB at the dose of 0.54 $J.cm^{-2}$.

The polyphenol anti-radical activity is linked to the presence of free hydroxyl functions (-OH).[9,10] The potentilla rhizome extract has a stabilised polyphenol form obtained by a process during which free phenolic functions are masked by esterification. During the cutaneous barrier passage, stabilised potentilla polyphenols are hydrolysed. The esters bonds are cleaved by esterases, enzymes naturally found on skin surface. Consequently, the masked phenolic functions are released. This results in an antioxidant potential.

The MDA level, or lipid membrane oxidation, is decreased by 38% in the presence of the potentilla extract (0.6 mg)

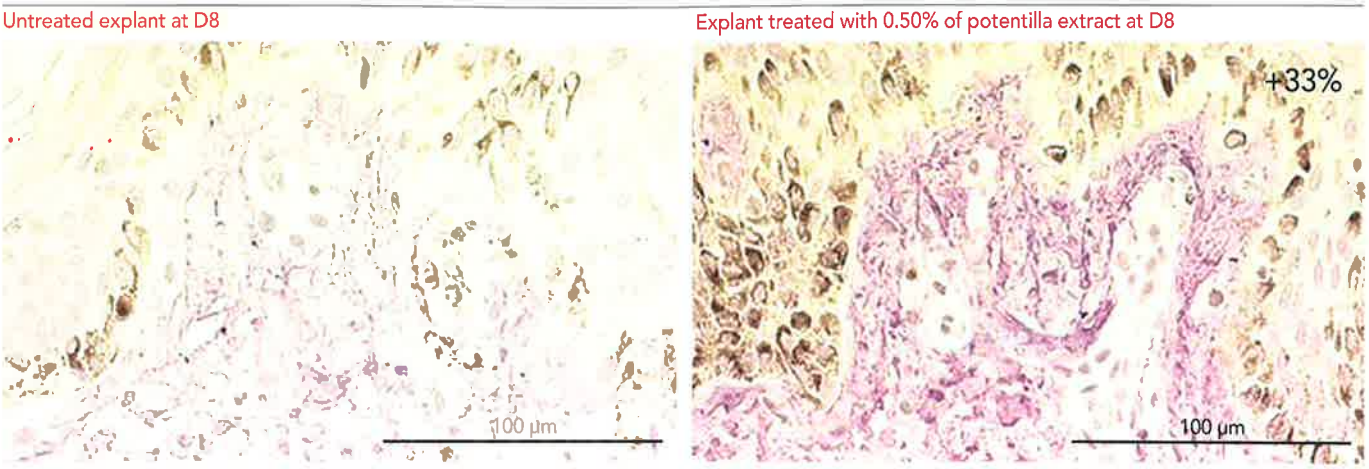


**Figure 2:** Stimulation of collagen III synthesis on skin explant at 0.50%.

compared to the untreated control after UV irradiation. This anti-radical activity is almost four times higher than the one obtained with vitamin E, a leading antioxidant substance.

### Collagen synthesis

Collagen, the most abundant protein in the dermis, plays a key role in maintaining skin structure. Organised in fibres, the different types of collagens create a three-dimensional network which crosses the dermis and brings resilience force and integrity to the skin. Cutaneous ageing is reflected in a loss of dermis density and tissue slackening due to declining collagen synthesis.

The effectiveness on the synthesis of collagen III was tested on skin explants, from a 46 year-old female donor, after eight days of treatment. The potentilla extract was used in concentrations of 0.25% and 0.50%. Activity was assessed by morphology observations with optical microscope and by image analysis after a specific immunolabelling of collagen III.

The results showed that the ingredient stimulates the synthesis of collagen III by 9% at 0.25% and by 33% at 0.50% compared to the untreated explant (Fig 2).

## Skin texture improvement: clinical studies

Linked to the ageing process, the structural and functional modifications which occur in the epidermis and in the dermis, make visible degradations on skin surface: , reduction in the quality of the skin's micro-relief and texture, increase in wrinkles, loss of firmness.

### Anti-glycation activity

Causes of skin ageing can be very different. Glycation phenomenon is one of them. This is a non-enzymatic reaction between sugars

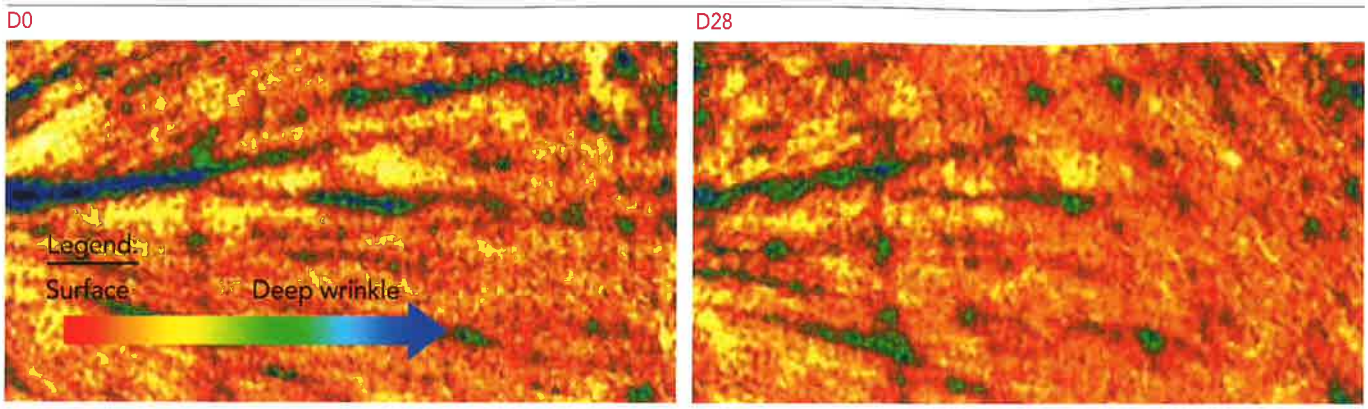


Figure 3: Anti-wrinkle activity of potentilla rhizome extract after 28 days of treatment at 0.50%.

and proteins, such as collagen or elastin. This spontaneous process leads to the formation of AGEs (Advanced Glycation Endproducts). Once glycated, proteins become rigid and lose their functions. AGEs induce oxidative stress through the formation of free radicals. The result of glycated protein and AGEs is premature skin ageing: the wrinkle formation is accelerated, skin loses its elasticity and firmness, finally the complexion appears dull.

AGEs become fluorescent under UVA irradiation. The measurement of AGEs quantity was performed thanks to the AGE Reader device by measuring their autofluorescence. There is a linear relationship between the skin autofluorescence and the age of volunteers. By calculating the difference between the theoretical age of the volunteer at D0 and the theoretical age at D28 obtained by measuring AGE autofluorescence, we can obtain a gain of X years of glycation.

The efficacy was evaluated on 23 female volunteers, aged 40 to 55, over 28 days. The ingredient was tested at 0.50% in formula versus a placebo and applied twice a day on the forearms. At the end of the study, the potentilla extract induced a significant reduction in the AGE autofluorescence value of 4%, which corresponds to a gain of 2.7 years of glycation. By fighting against the glycation effects, the skin is firmer and the complexion more radiant.

Anti-glycation is a common subject in the cosmetic field. However, the main existing tests require *in vitro* or *ex vivo* studies. Here, the research leads us to determine the anti-glycation activity of a product with clinical analysis.

### Anti-wrinkle activity

The effectiveness of the potentilla rhizome extract was evaluated on the wrinkle formation. In fact, the protection of skin against free radicals and glycation, and the stimulation of collagen synthesis, may limit the visible consequences of the ageing process such as wrinkles.

A 28 day study was conducted on a panel of 22 female volunteers with slack skin, aged of 40 to 55, with twice daily application. The results from the evaluation, at 0.50% of active ingredient, were compared to placebo. The anti-wrinkle action was measured in the crow's feet area. Using fringe projection – performed by PRIMOS measurement system, the three parameters of the skin relief were studied. The first one is average roughness, which characterises the micro-relief and texture of the skin, the other ones are average depth and maximum surface area which characterise deep wrinkles.

At the end of the 28 day period, thanks to the potentilla rhizome extract, the wrinkles were significantly reduced (Fig 3). Skin roughness, wrinkle depth and wrinkle surface area are decreased. The ingredient targets both the micro-relief and texture on the surface of the skin and deep wrinkles. The placebo had no effect.

## Conclusion

Ageing is a well-studied cosmetic claim. However, it is still a complex and specific phenomenon. Despite the number of studies, its mechanism of action is not fully understood and its effects are not completely explained as well as its causes. As a single, unique theory of ageing does not exist, many research areas remain essential to complete our knowledge on active ingredients claiming an anti-ageing property. The elaboration and study of this potentilla extract is part of one of them. Ageing is not just a matter of visible signs on the skin surface, in-depth consequences are quite as important. Anti-ageing active ingredients have to take action on both levels: cellular and skin surface.

Berkem's research reveals the potential of an un-used plant in cosmetics, the potentilla, on ageing fields thanks to a specific method to stabilise polyphenols. It acts throughout the dermis and epidermis by stimulating collagen synthesis, preventing from free radical and glycation effects. As a result, it improves the overall skin surface, in particular micro-relief and deep wrinkles, as well as its texture and quality. The skin is smoother, firmer and looks younger. The technology applied brings a liposoluble characteristic and heat resistant property to the active ingredient.

Finally, the active ingredient has been recognised by two innovation awards (the Advanced Ingredient Award during the Beyond Beauty Lab in 2015 and the third place in the category 'Natural products – most innovative raw material' during the BSB Innovation Awards ceremony in 2016). PC

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