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Assoc. Prof. Dr. Nina Kocevar Glavac,
Editor in Chief

Dear Readers,

The Modern CosmEthics association stepped into the world of cosmetic science back in 2018 when we published the Modern Cosmetics book. It is tremendous recognition for us that it has become one of the world's leading publications in this field. It is now present in **93 countries** across the world!

Our educational activities didn't end there. Three years later, the first volume of the **CosmEthically ACTIVE Journal** has seen the light of day. With the journal, we continue to follow our main goal of bringing the facts closer to you. It is not only a relevant, state-of-the-art source of literature about cosmetic science, but also an inspiration for building and improving your own cosmetics brand.

So, today the CosmEthically ACTIVE Journal goes on! This volume presents:

- Three popular yet controversial ingredients: **bakuchiol**, **resveratrol** and **hyaluronic acid**. *Are they worth the hype?*
- Four chemically complex plant-based substances: **CO_2 extracts**, **unsaponifiable compounds**, **plantain extracts** and **anti-inflammatory ingredients**. *They show a highly relevant potential for use in active cosmetics.*
- Two sustainable and environmentally-friendly concepts in modern formulating: **responsible cleansing** and an evidence-based procedure for the **extraction of grapes** using glycerol and water. *All of us can contribute to better formulating practices.*

Last but not least, I would like to **sincerely thank** the companies that financially supported the release of the CosmEthically ACTIVE Journal 2022. We have recognised them as trustworthy partners who offer materials and services of the highest quality. I kindly encourage you to get to know and establish contact with them.

In the end… We have always been a small team of motivated researchers focused on the concepts of **evidence-based science and green technologies** in the area of medicinal plants. The recognition of our goals and professional competencies gives us additional motivation to promise you:

We will continue working for you and with you!

Sending you CosmEthically ACTIVE wishes,

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Responsible cleansing

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ABSTRACT

Surfactants are considered to be among the most important cosmetic ingredients, as they are used for cleansing, thickening, stabilising, solubilising, emulsifying and foaming. However, surfactants also exhibit unwanted effects: skin irritation. The concept of responsible cleansing with respect to the skin, hair and the environment using surfactants with less irritation potential has taken a leading role in modern formulating.

Keywords: CosmEthically ACTIVE, natural cosmetics, skin irritation, surfactants

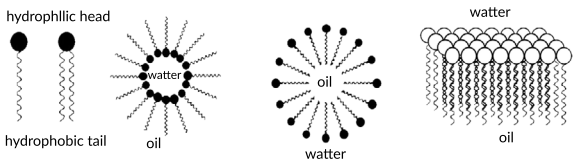
Article category: Professional article
Presern A, Kocevar Glavac N. Responsible cleansing. Cos ACTIVE J. 2022;2:4–8

FOR STARTERS: SURFACTANTS AND THE VERSATILITY OF THEIR USE

Surfactants or surface-active substances are used in many industrial sectors, primarily as a base for cleaning products. They are found, for example, in dishwashing gels, laundry detergents and car washes. Even when we focus on cosmetics, surfactants are considered to be among the most important cosmetic ingredients, as they are used for **cleansing, thickening, stabilising, solubilising, emulsifying and also foaming** alone (1–3).

STRUCTURE DEFINES PROPERTIES

Surfactants are **amphiphilic** or bipolar compounds, which means they contain both polar (hydrophilic) and non-polar (hydrophobic) groups. Due to this specific structure, surfactants are soluble in both polar and non-polar solvents. Or to put it simply, they are compatible with both water and oil, which accounts for the majority of their applications. They can be 'absorbed' at the interfaces between phases of different polarity and therefore lower the interfacial tension in systems of two non-miscible liquids, or lower the surface tension of water at the interface with the air (1–5).



In a solution, surfactants firstly self-organise at the interface. As their concentration in a solution increases, and all the places at the interface are already occupied, an increasing number of surfactant monomers are present in the solution. To minimise their movement and interactions and, most importantly, to reduce the surfactant contact surface with solvent molecules, the system tends to stabilise itself by combining monomers into aggregates called **micelles** (1–3).

The concentration at which this is spontaneously achieved is characteristic for each surfactant, and is referred to as critical micelle concentration. Surfactants in micelles have their hydrophobic tails aligning with non-polar solvent (lipids) and their hydrophilic heads aligning with a polar solvent (water) (1–3, 5). With regard to cleansing the skin or the scalp and hair, where we aim to remove sebum and environmental dirt, surfactants can solubilise in micelles, which are later washed away by water (2–5).

SURFACTANTS AND THE SKIN: REACTIONS

In addition to this desired effect of providing clean skin, cosmetologists and dermatologists have also focused their attention in recent years on the undeniable adverse skin effects of surfactants. The use of hair and body wash cosmetic products and household detergents is actually considered one of the primary causes of **skin irritation**. But that fact may not be so surprising if we simply consider how many times each day our skin is exposed to them. Surfactants can be adsorbed on the skin surface, interact with both proteins and lipids in the stratum corneum, and if they penetrate deeper, they may even damage the skin layers below (1, 3–5).

Surfactants primarily exhibit their unwanted effects in the outermost skin layer, the stratum corneum. They can bind to **stratum corneum proteins**, such as keratins, which can result in protein denaturation, leading to swelling of the stratum corneum and the accelerated washing away of proteins from the skin. Consequently, chemicals and pathogens from the environment may penetrate deeper into the skin more easily, causing immune responses, seen as itching and red patches. Swollen proteins also bind less water, so the skin becomes less moisturised and less flexible (1, 3).

Historically, the concentration of surfactant monomers in solutions was believed to be the greatest factor of surfactant-caused skin irritation. This was the result of a commonly recognised model of interactions between surfactants and stratum corneum constituents, known as a 'surfactant monomer skin penetration model', where only small-sized monomers were thought to be able to penetrate into this skin layer and interact with proteins. It was believed that after the **critical micelle concentration** of a surfactant in a solution was reached and the micelles

were assembled, the severity of surfactant irritation was reduced (1, 5).

However, studies have shown that even the application of products in which the critical micelle concentration was exceeded caused skin irritation and that the negative effect increases with a higher surfactant concentration in a solution (even though the monomer concentration is constant above the critical micelle concentration). Because micelles are unstable aggregates, they are able to disintegrate into monomers following contact with the skin, after which small micelles are formed. All of this led to the conclusion that both monomers and micelles have the ability to cause protein denaturation. Later research also linked surfactants that form smaller micelles to an increased skin irritation effect (1, 5).

Another investigated mechanism of skin irritation caused by surfactants was their effect on **lipids in the intercellular matrix of the stratum corneum**. Again, both surfactants in monomer or micelle form can exhibit adverse effects. Surfactants at or above the micelle-forming concentration have the ability to solubilise stratum corneum lipids into micelles and wash them away with water, just as is desirable with sebum, sweat and dirt on the skin surface. This leads to stratum corneum (uneven) delipidation, resulting in the weakening of skin barrier function, seen in increased transepidermal water loss, excessive skin stiffness, dry skin, cracking and erythema (1, 3).

Some studies have also shown the ability of monomers to become embedded into the intercellular matrix of the stratum corneum, which increases permeability and enables monomers to penetrate even deeper into the skin, intensifying irritation. Embedded monomers also damage the enzymes responsible for production of intercellular matrix components (1, 5).

The skin barrier function is impaired due to the two above-described processes. Damaging agents are thus able to penetrate easily into deeper epidermal skin layers, where they can damage keratinocytes. Surfactants can impair keratinocytic cytoplasm and proteins, which can both lead to permanent cell damage or even cell death (1, 3).

The presence of surfactants can also cause the release of **inflammatory mediators** from cells, inducing

dermal inflammatory reactions. The most common reaction is irritant contact dermatitis, where the inflammatory process is only present at the site of contact with the irritant, resulting in red skin, scaling, erythema and sensations of pain, discomfort and itching. Another inflammatory reaction is allergic contact dermatitis, where the immune response recognises a surfactant as an allergen. This results in an inflammatory response on the entire surface of the skin, causing bullae, redness, oedema and itching (1, 3).

UNDERSTANDING REACTIONS: SURFACTANT CHEMISTRY

The irritation effect of surfactants is closely linked to their chemical structure. If a surfactant dissolves in water due to the formation of hydrogen bonds with water molecules, it is classified as non-ionic. **Non-ionic surfactants** form weaker bonds and are therefore less capable of causing skin irritation. If a surfactant undergoes electrolytic dissociation in water, it is categorised as ionic. **Ionic surfactants** are further classified as anionic or cationic, and if a surfactant carries both a negative and positive charge, depending on the pH, it is called **amphoteric**. Due to strong electrostatic interactions with the skin's proteins and lipids, ionic surfactants (the most well-known example being sodium lauryl sulphate) are primarily attributed to the aforementioned adverse interactions (1–3).

The second parameter important for affecting the barrier function is the **length of alkyl chain**. An increase in skin irritation, shown by measurements of transepidermal water loss, was detected when the length of alkyl chains is increased, but only up to C12 (again, the characteristic of sodium lauryl sulphate), and then decreased with C14 and C16 surfactants. This is due to an increase in the surfactant's lipophilicity, which in turn decreases the affinity to stratum corneum proteins (1–3).

Thirdly, irritation potential also depends on the **size of a surfactant's polar part** with a given alkyl chain length. Larger polar parts cause less damage and irritation due to a reduced negative charge density, thus producing weaker electrostatic interactions with proteins (1, 6).

IN THE SPOTLIGHT: HOW TO MINIMISE NEGATIVE EFFECTS

As explained, surfactants provide effective cleansing, but are also able to remove skin structural components and damage the barrier function, causing subclinical or clinical skin conditions. It is therefore of the utmost importance to select cleansing products with ingredients that respect our skin.

New generations of surfactants, which also possess better characteristics in terms of sustainability and biodegradation, are becoming increasingly available and used, e.g. alkyl (poly)glucosides and amino-acid based surfactants, such as alkyl glutamates, glycinates, alaninates and sarcosinates (6, 7). They also offer innovative formulating opportunities, such as so-called waterless and water-efficient cosmetics (8).

Nevertheless, keep in mind that the vast majority of cleansing products and household detergents on the market still contain **sodium lauryl sulphate**, which has been proven to be one of the most irritating surfactants. Due to its superior solubilisation power and strong effect on cell components, it is often used in biochemical gene research, as well, as a cell lysis reagent and in dermal studies as an irritation agent (3–5). Yet, we continue to apply massive amounts to our skin!

EVIDENCE-BASED FORMULATING

In order to formulate a cleansing product with less irritation potential, conscious formulators try to reduce the critical micelle concentration of the overall surfactant system, which leads to less monomers in the solution, and an increase in micelle size and improved stability. This could be achieved by selecting surfactants that have proven to cause less irritation (such as non-ionic surfactants or surfactants with larger polar heads), or by adding cosurfactant(s) to the system (1, 5).

Because the concept of the **CosmEthically ACTIVE certificate** focuses on evidence-based activity and skin compatibility, we do not permit the most irritating surfactants, such as sodium lauryl sulphate, ammonium lauryl sulphate and sodium coco sulphate.

The overall composition of every CosmEthically ACTIVE certified cosmetic product must follow parameters of both the skin's and hair's natural physiology. We therefore attribute great importance to the pH value of formulations, as well. The appropriate pH level of the skin surface is crucial for an undisturbed skin barrier function, and the altering of that function disrupts the normal synthesis of enzymes responsible for the production of intercellular matrix components and their activity, and causes the dysbiosis of the **skin's microbiota**. For this reason, we also do not permit the use of solid soaps obtained by saponification due to the high pH they leave after application to the skin during washing. To guarantee that CosmEthically ACTIVE certified products meet the highest level of environmental protection, surfactants obtained by alkoxylation, such as cocamidopropyl betaine and cocoamphoacetate, are also not permitted (9).

CONCLUSION

An increase in consumer awareness has spiked the demand for natural cosmetics and natural surfactants. Indeed, where there is demand, there is always supply: an increasing number of products available on the market contain natural surfactants with less potential to provoke adverse interactions with the skin's components, which is of the utmost importance given the frequency of their use. New generations of surfactants of natural origin are both skin- and environment-friendly, and demonstrate excellent dermal compatibility and biodegradation.

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Bakuchiol: A promising ingredient to help slow down time

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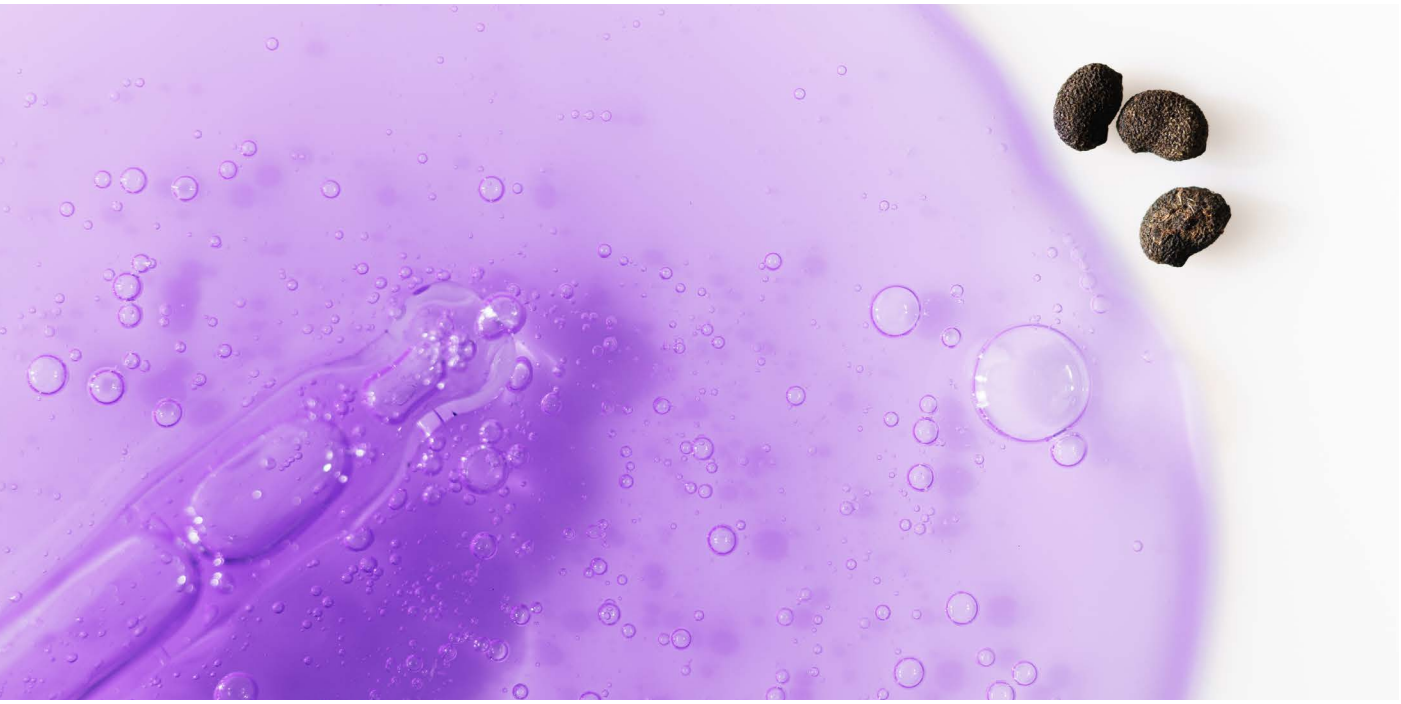
ABSTRACT

Bakuchiol, a phytochemical found in the plant *Cullen corylifolium*, exhibits similar effects on gene expression profile as retinol and therefore acts as its analogue, improving wrinkles and uneven pigmentation, increasing skin firmness and correcting overall skin quality without significant undesirable side effects, such as irritation, dryness and sensitivity.

Keywords: bakuchiol, clinical study, cosmetics, retinol



Article category: Scientific review article
Presern A, Kocevar Glavac N. Bakuchiol: A promising ingredient to help slow down time. Cos ACTIVE J. 2022;2:9–15

INTRODUCTION

The cosmetics industry has always been on a never-ending quest to find a magic wand and slow the skin's biological clock or even turn it back. Among such promising cosmetic ingredients, bakuchiol is gaining more and more interest as a natural alternative to the holy grail of anti-ageing, retinol, but without the latter's well-known side effects, such as burning, stinging and erythema.

During the **ageing process** or due to chronic sun exposure, for example, our skin becomes thinner, loses elasticity, and develops wrinkles, uneven pigmentation and textural irregularities. As this, apart from the strictly cosmetic point of view, leads to poor wound healing and increased susceptibility to skin problems and diseases, we can say that this is also becoming an important public health issue in the context of an ageing population (1, 2).

Topical retinoids are widely used today in cosmetic products for the reduction of the signs of ageing and photodamage. Their ability to 'fight time' has been clinically proven, for example, through the stimulation of the production of collagen and glycosaminoglycans, known to bind a substantial amount of water, accelerate cell regeneration and alter melanin synthesis, which can all be seen as an improvement in fine lines, wrinkles and uneven pigmentation (1–3).

However, despite these desirable results, the retinoid application is often accompanied by significant **undesirable dermal effects**, such as skin irritation, dryness, erythema, pruritus, scaling, burning and/or a stinging sensation, all of which restrict their use, especially in subjects with sensitive skin (1, 2, 4, 5).

WHAT IS BAKUCHIOL: THE PROPERTIES

Bakuchiol is a phytochemical from the group of **phenylpropanoids** with the chemical formula 4-[(1E,3S)-3,7-dimethyl-3-vinylocta-1,6-dien-1-yl]phenol (Figure 1). It is found mainly in the seeds and leaves of ***Cullen corylifolium*** L. Medik, formerly known as ***Psoralea corylifolia*** L. and also called Babchi or **Bakuchi**, hence the compound name (2, 6). It has also been isolated from other plants, such as *Psoralea granulosa* (7), *Ulmus davidiana* (8), *Otholobium pubescens* (9) and *Piper longum* (10). Bakuchiol is valuable in both cosmetic science and (traditional) medicine, and has been used widely for centuries in Indian and Chinese medicines to treat a variety of conditions. The dry fruit of *C. corylifolium* also has monographs in both Chinese and Ayurvedic Pharmacopoeias (6).

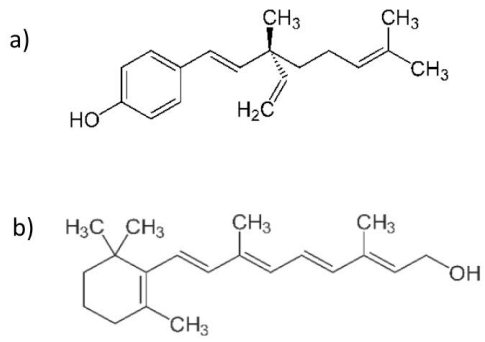


Figure 1: Chemical structures of bakuchiol (a) and retinol (b).

Bakuchiol comes in the form of a **viscous colourless to yellowish oil**, soluble in a variety of organic solvents (e.g. ethanol and DMSO; DMSO is only relevant for scientific research) and lipid emollients, such as vegetable oils and capric/caprylic triglycerides. It is only sparingly soluble in an aqueous media. It shows **good stability** under recommended storage conditions. Bakuchiol as a cosmetic raw material should be stored at temperatures up to 30 °C. It should not be exposed to heat and light (11–13).

IN VITRO STUDIES

In terms of function, bakuchiol acts as retinol's functional analogue, which is also supported by their structural similarity (Figure 1). Chaudhuri et al. showed in 2014 that both retinol and bakuchiol exhibit similar **effects on the gene expression profile**, obtained in an *in vitro* full-thickness human skin model (4). This includes the modulation of retinoic acid receptor genes and genes implicated in the formation of an extracellular matrix and dermo-epidermal junction constituents (4).

Apart from these retinol-related pathways, bakuchiol induces additional ones that may contribute to its anti-ageing effect, most notably **antioxidative** processes (14, 15). Many other actions of bakuchiol have also

been reported, such as **anti-inflammatory** (16, 17), which may contribute to a better side-effects profile, **anti-viral** (18), **anti-tumour** (19), **antibacterial** (20), **antiacne** (21, 22) and **skin-lightening** (10).

CLINICAL STUDIES

Previously conducted scientific studies have provided evidence for the wide range of biological activities of bakuchiol. However, we will focus on the ones that studied **anti-ageing effects and a comparison with retinoids**.

The effects of a skincare product containing bakuchiol were studied in a clinical study where 16 photoaged women used a 0.5% bakuchiol cream twice a day (morning and evening applications to the entire face). After twelve weeks of treatment, clinical assessment of the skin showed improvement in facial fine lines and wrinkles, pigmentation, firmness and elasticity, and an overall reduction in photodamage. Profilometry using silicone replicas indicated a significant reduction in wrinkle depth compared to the baseline. It should be emphasised, however, that the study lacks a vehicle control (4).

To compare the clinical effectiveness and negative skin reactions between bakuchiol and retinol, a randomised, double-blind, 12-week study was conducted, focusing on treating common signs of skin ageing (2). It included 44 subjects, divided into two groups, using either a 0.5% bakuchiol cream twice daily (19 females and two males) or a 0.5% retinol cream nightly (22 females and one male), both formulated in the same vehicle, on the entire face. The application differences were consistent with typical products' regimens and, considering bakuchiol, reflect its well-tolerated daytime use without photosensitivity. Results showed no statistical difference between the effects of bakuchiol and retinol. Both preparations significantly decreased fine wrinkle surface area and improved skin hyperpigmentation compared with the baseline. Subjects from the retinol group, however, reported significantly more scaling and stinging. Bakuchiol was observed to show more redness at week 4, but the difference in redness compared to retinol was no longer significant at weeks 8 and 12. Researchers concluded that the anti-ageing effect and good tolerability of bakuchiol were confirmed in the study. However, it is important to emphasise that the study blinding design is questionable. The application regimen of the bakuchiol group (twice daily) was different than that of the retinol group (once daily), but researchers did not explain how the blinding approach was provided.

Another study (23) included 60 female subjects with mild to moderately photodamaged skin and self-perceived sensitive skin, who used two products containing bakuchiol (a cleanser and a moisturiser), twice daily (mornings and evenings) for four weeks. The moisturiser contained 1% bakuchiol, while the content in the cleanser was not specified. The aim of this research was to examine tolerability, skin barrier effects and the effectiveness of bakuchiol on sensitive skin. The researchers did not identify any tolerability issues. Some 10% of subjects with eczema reported minor stinging, while a few subjects observed mild tightness. Overall, the subject- and researcher-assessed tolerability was deemed to be excellent, as 1/3 of subjects had eczema or atopic dermatitis, rosacea and cosmetic intolerance syndrome, respectively. Improvements in smoothness, clarity and radiance, and the anti-ageing effect and overall appearance were statistically significant, as determined by researchers. TEWL (transepidermal water loss) measurements showed no changes from the baseline over the four weeks of treatment, which reflects no damaging effects on the skin barrier. Corneometry measurements showed a significant increase in skin moisture content. However, the study was not blinded, nor did it include a control.

The studies described below were conducted on products containing bakuchiol in combination with other anti-ageing ingredients. In the work by Goldberg et al. (24), five clinical studies were performed with a duration from four to 12 weeks and a total of 103 subjects with different skin types using a night serum with bakuchiol (0.5%), melatonin (0.1%) and ascorbyl tetraisopalmitate (10%) once daily. Two randomised studies, each on 24 subjects, focused on skin hydration following the one-time application of a serum on the forearm skin. A significant hydration improvement was reported over 12 hours, while a decrease in TEWL was significant until the peak at six hours. The effectiveness and tolerability study in-

cluded 39 females with moderate skin ageing and at least one pigmented spot on the face, who applied the serum each evening for three months. Instrumental and visual evaluation (performed by a dermatologist or the subjects themselves) confirmed beneficial effects, including significant improvements, such as a decrease in wrinkles, an increase in skin firmness, skin lightening, a reduction in redness and overall improvement in skin quality. In the fourth study, the serum was tested on the oily skin of 31 females with signs of ageing. After 28 days of application every evening, sebum secretion decreased significantly. Subjects in the fifth study (33 females with oily or combination skin with comedones) had fewer comedones after 28 days of use. The serum was thus confirmed as non-comedogenic. The serum was well tolerated. The main limitations are a lack of a placebo and blinding.

The same research group continued the examination of the same product on 24 subjects, with a focus on additional, detailed clinical and histologically confirmed effects (25). An overall improvement in wrinkles, photodamage and hyperpigmentation was confirmed after nightly application over a 24-week period. The most favourable histological results after skin biopsy on five subjects included increased dermal and epidermal thickness, and a significantly increased level of collagen III. The same limitations as the above-mentioned apply to this study.

These are some of the recent clinical studies conducted that show bakuchiol's retinol-type functionality, without retinol-like unwanted skin reactions. Nevertheless, **further studies of longer periods, on expanded populations and with better study designs are needed to show the clear, evidence-based clinical effects of products containing bakuchiol**.

SAFETY PROFILE

Even though both the topically applied retinoids and bakuchiol typically express marked improvements in various anti-ageing skin parameters, there are a few major advantages of bakuchiol over retinol (and its derivatives). These include better **skin tolerability, stability and safety** profile (2, 4, 23–25).

In terms of adverse skin reactions observed in the study by Dhaliwal et al. (2), for example, significantly more scaling and stinging were reported for retinols, while redness was more pronounced in the bakuchiol group, although not at significantly higher levels compared to the retinol group.
Along with the increased use of cosmetic products containing bakuchiol as a relatively new ingredient, individual **case reports about irritation reactions** in the form of contact dermatitis have become available. Positive reactions in patch tests were confirmed for 0.1% bakuchiol in a 33-years old female (26) and for 1% bakuchiol in a 23-years old female (27).

EVIDENCE-BASED FORMULATING

Scientific research of bakuchiol's effects, summarised in the *'In vitro* studies' section (6, 8, 10, 14–20), intensified after 2007 when bakuchiol was introduced to the market as a cosmetic ingredient under the trade name Sytenol® A by Sytheon (28). According to CosIng, the European Commission database for information on cosmetic substances and ingredients, bakuchiol is classified for cosmetic use due to its antimicrobial, antioxidative, skin conditioning and emollient properties (29).

Based on data from the presented studies and specifications of bakuchiol ingredient manufacturers (13, 28), **recommended bakuchiol concentrations in finished formulations range from 0.5 to 1%** (w/w), the former being the most commonly evaluated in clinical studies and demonstrating good cosmetic activity (2, 4, 24, 25).

Based on data from the presented clinical studies (2, 4, 24, 25). and specifications of bakuchiol ingredient manufacturers (13, 28), **recommended bakuchiol concentrations in finished formulations range from 0.5 to 1%** (w/w), the former being the most commonly evaluated in clinical studies and demonstrating good cosmetic activity.

Many different skin types may benefit from its use, including subjects with normal, oily, combination, dry and even sensitive skin. **Sensitive skin** is especially prone to irritation when using topical retinoids and could therefore find anti-ageing products with bakuchiol as a more suitable alternative. Above all, **aged and photoaged skin** may benefit most from the use of cosmetics containing bakuchiol (24, 25). Unlike retinoids, bakuchiol can be used during daytime due to its **photostability** and may also stabilise retinol to some degree when used together (2, 4).

Bakuchiol shows **miscibility with a wide variety of lipid emollients**, such as capric/caprylic triglycerides and vegetable oils, alkyl benzoates (C12-15, C16-17), mineral oils and silicones (dimethicones, cyclomethicones). Please note that some are not accepted in terms of the concept of natural cosmetics. Bakuchiol is suitable for incorporation into emulsion systems and lipid solutions, such as oil serums. In order to incorporate it into an emulsion, it must be separately dissolved in a lipid emollient/vehicle and added to the emulsion at a temperature of approximately **50 °C or below**. If not exposed to higher temperatures for a longer period of time, it can be added directly into the lipid phase. An **acidic pH below 6.5** should be maintained. In addition, bakuchiol is **not compatible** with metal ions, such as iron or copper, due to a consecutive colour formation, as it is a phenolic compound. However, chelators may be used to avoid colouration issues (22).

Finally, formulators need to be aware of the fact that other bakuchiol-containing or Babchi plant extract-containing ingredients are available on the market. They typically contain furanocoumarins (also known as furocoumarins) such as psoralen, which induce phototoxic skin reactions (30).

CONCLUSION

Because it exhibits similar effects on the gene expression profile, bakuchiol shows retinol-like anti-ageing functionalities. Most importantly, they are delivered without significant unwanted skin reactions related to the typical use of retinoids, especially in sensitive skin. Further clinical studies of good methodological quality are undoubtedly needed to confirm clinical effectiveness. We can, however, conclude that even though bakuchiol is not a magic wand to slow down time, it is surely a great rival of retinoids in terms of younger, smoother and plumper skin.

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Resveratrol: An antioxidant for attractive beauty applications

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ABSTRACT

The skin is exposed to many harmful factors. To remain healthy and good-looking, and to be able to resist or recover, balanced regeneration mechanisms are of great importance. One of these is called antioxidant capacity. What happens if the process of removing free radicals becomes saturated and the skin unbalanced? In this scenario, it is extremely beneficial to introduce antioxidants dermally. Resveratrol, a substance found in grapes and red vine, has been proven *in vitro* and clinically to have a high antioxidative strength. Not only does it play a major role in protection against UV radiation, it also demonstrates significant positive skincare effects in many skin conditions such as acne, wrinkles and pigmentation spots.

Keywords: antioxidant, resveratrol, skin, UV radiation

Article category: Scientific review article
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INTRODUCTION

Skin layers, especially the epidermis and dermis, are exposed to degradation due to ultraviolet (UV) radiation. The matrix of collagen, elastin and hyaluronic acid fibres is responsible for the elasticity and firmness of the skin. Photo-induced, oxidative or enzymatic degradation of the skin's structures can lead to **premature skin ageing and photo-ageing**, inflammation and other photo-induced diseases, including melanoma and non-melanoma skin cancers (1).

In terms of cosmetic applications, **antioxidants of natural origin** represent one of the most important approaches to counteract the damaging effects of oxidative processes. They act on the skin surface and can also penetrate through the skin barrier, and decelerate ageing. As a part of this group, polyphenols are plant metabolites with many bioactive properties. Besides their action as free radical scavengers, they possess anti-collagenase, anti-elastase and anti-hyaluronidase activities as the most significant skin-relevant effects (1).

Different types of **polyphenols** exist, including simple phenols, phenolic acids and flavonoids (1). A rising polyphenol for beauty applications is **resveratrol**, a stilbenoid or hydroxylated derivative of stilbene in chemical terms. It has gained interest as a cosmetic ingredient because of its antioxidative, anti-inflammatory and antiproliferative activities at the molecular level, and antiwrinkle, photo-protective and skin-whitening properties at the sensory level, to summarise a few of its cosmetic effects (2, 3).

ANTIOXIDANTS OF NATURAL ORIGIN IN COSMETICS

Antioxidative compounds are used to protect human skin against damage caused by **free radicals**, including those that arise from UV radiation (4). To maintain healthy, normally functioning skin, the oxidative and antioxidative processes in both the skin and the whole body must be in balance.

Antioxidants delay, prevent and eliminate oxidative damage. There are many positive effects on skin health related to this mechanism of action, such as diminishing the formation of visual signs of ageing and boosting the (skin) immune system (5).

In cosmetic formulations, natural antioxidants are used as a single, pure compound or a mixture of compounds, both as natural isolates or synthetically produced, i.e. nature-identical compounds, or as plant extracts. They typically function as quenchers of reactive oxygen species or enzyme inhibitors (4).

Due to their instability, concentrations of each antioxidant must be controlled and stability-tested to ensure that a cosmetic product expresses and maintains the claimed activity. This is of particular importance in cosmetic science research and within the concept of active cosmetics. Antioxidative compounds of plant-derived extracts, for example, are very complex. It is thus difficult to determine their activities.

Some of the natural antioxidants used in cosmetic products are resveratrol, which is the focus of this article, α-tocopherol and natural tocopherol mixtures (vitamin E), ascorbic acid (vitamin C), β-carotene and other carotenoids, curcumin, quercetin and other flavonoids, etc. (4).

RESVERATROL AS A COSMETICALLY ACTIVE INGREDIENT

Resveratrol found its place in scientific history back in 1992, in connection with food consumption habits popularly known as the "**French paradox**". The paradox refers to a paradoxical epidemiological observation that French people have a relatively low incidence of coronary heart disease while having a diet rich in saturated fats. This was first explained to be related to alcohol (wine) consumption and later to resveratrol in red wine, which is commonly used in the French diet (6).

Resveratrol has been found in almost **70 plant species** such as grape vine (*Vitis vinifera*), peanut (*Arachis hypogaea*), acai (*Euterpe oleracea*), raspberry (*Rubus idaeus*), Japanese knotweed (*Reynoutria japonica*, formerly known as *Fallopia japonica* and *Polygonum cuspidatum*), etc. The molecule is biosynthesised in plants as the **mechanism of resistance to stressful factors**, including fungal or parasite infections, UV radiation and chemical substances (5, 7).

Resveratrol is a 3,4',5-trihydroxystilbene (Figure 1) with a chemical formula of $C_{14}H_{12}O_3$ and a molecular weight of 228.24 g/mol (3). It appears as white or almost white powder practically insoluble in water. It is **soluble** in ethanol, caprylic/capric triglyceride and alkanediols, such as 1,2-hexanediol, pentylene glycol or propanediol (8). Also, it is soluble in dimethyl sulfoxide and acetone (7), but these reagents are only relevant for scientific research.

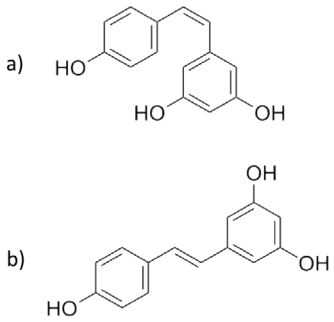

Figure 1: Chemical structures of cis-resveratrol (a) and trans-resveratrol (b).

Furthermore, resveratrol exists in two isomeric forms, *cis* and *trans* (Figure 1); the latter is the **biologically active form**. *In vitro* studies have shown that the *trans*-form is more photo- and thermostable than *cis*-resveratrol. The isomerisation of *trans*-resveratrol to *cis*-resveratrol is influenced by exposure to sunlight or UV irradiation. Some factors that affect this process are irradiation time and wavelength, temperature and pH, and the physical status of the molecule (i.e. solid or in a solution). No increase of *cis*-resveratrol typically occurs at 50 °C if the pH is maintained below 7 and the formulation is kept away from sunlight (9).

In the cosmetics industry, resveratrol (INCI: *Resveratrol*) is mostly used as a pure compound with a **recommended concentration** of 0.1 to 1% (8). Cosmetic labels may also include ingredients that contain resveratrol such as grape extracts (INCI: *Vitis Vinifera Vine Extract* and *Vitis Vinifera Leaf Extract*), products obtained through the fermentation of *Pichia* yeasts (INCI: *Pichia/Resveratrol Ferment Extract*), or filtrates of products obtained through the fermentation of resveratrol and plant extracts using *Lactobacillus* bacteria (INCI name: *Lactobacillus/Camellia Sinensis Catechins/Gelidium Crinale/Laminaria Japonica/Monostroma Nitidum/Resveratrol Ferment Filtrate*). Resveratrol's derivatives are also used under INCI names such as *Hydroxyresveratrol*, *Glucosyl Resveratrol*, *Resveratryl Glucoside*, *Resveratryl Triacetate*, and dimethyl, tributyl and tripentyl ether of resveratrol (INCI: *Resveratrol Dimethyl Ether*, *Resveratrol Tributyl Ether*, *Resveratrol Tripentyl Ether*), and others (3).

BIO-PRODUCTION OF *trans*-RESVERATROL

The increased demand for resveratrol for cosmetic and pharmaceutical applications requires production from sustainable sources (10).

On an industrial scale, the extraction and purification of *trans*-resveratrol from plants remain challenging, expensive and in some cases even incompatible with sustainable approaches. On the other hand, the chemical synthesis of resveratrol leads to difficulties in the purification of the ingredient and may be hazardous when the compound is intended for use in food or medicine (11).

Research has therefore focused on the **fermentation process**. Typically used hosts for resveratrol biosynthesis include *Escherichia coli*, *Lactococcus lactis*, *Streptomyces venezuelae* and *Corynebacterium glutamicum* as prokaryotes, and the yeast *Saccharomyces cerevisiae* as a eukaryote. To obtain a high-quality product, it is crucial to select a suitable host organism and to optimise the fermentation process (11).

The main advantages of **bacterial hosts** are a short life cycle, easy genetic manipulation and handling, a high level of enzyme and protein expression, and a high growth rate. On the contrary, the main disadvantages are the poor expression of large proteins and the low ability of so-called post-translational modifications (11). Post-translational modification is a special process during protein biosynthesis in every living cell and involves, for example, chemical changes and the formation of a native three-dimensional structure, in biochemistry also known as protein folding. Post-translational modifications are crucial for the functional activity of proteins.

The general advantages of the *Saccharomyces cerevisiae* **yeast host** are the intact ability of post-translational modifications and the good expression of large proteins, while the yeast is also easy to grow and genetically manipulate, and has a so-called generally recognised as safe (GRAS) status. The main disadvantage is a lower yield (11).

A brief overview of biosynthetic resveratrol production with yeast as a host starts with glucose, ethanol or glycerol as substrates, which are deemed to be of high sustainability grade. Other fundamental constituents for resveratrol's biosynthesis are the amino acids tyrosine and phenylalanine, and malonyl-CoA. Each of these compounds undergoes various reactions with the ultimate step involving the condensation of three units of malonyl-CoA with *p*-coumaroyl-CoA through an enzyme, stilbene synthase (10, 11).

Despite intensive research, including significant strain engineering, resveratrol production is still considered low. Ibrahim et al. report that resveratrol yield obtained using yeast fermentation reaches no more than 1 g/L of resveratrol and is significantly below industrial needs. They also stress that the path toward sustainable industrial production leads through even more intensive research and the deepening of scientific knowledge (10).

ANTIOXIDATIVE POWER OF RESVERATROL

Air pollution, cigarette smoke, UV radiation and psychological stress are some of the most prevalent harmful factors that lead to oxidative stress in human cells (12). **Oxidative stress** is an imbalance between the production of free radicals and the antioxidant defence system, and leads to tissue damage. Resveratrol has attracted a great deal of attention as an antioxidant, not only because of its protection power but also because it can be used in various applications, including cosmetic, food and even medical.

The **antioxidative power of resveratrol** is closely linked to its chemical structure composed of hydroxyl groups on benzene rings and conjugated with a double-bond ethene system (Figure 1). Research has confirmed that replacing the hydrogen atom in hydroxyl groups with a methyl group ($-CH_3$) or removing the hydroxyl group leads to a significantly reduced antioxidative activity, whereby the 4′ hydroxyl group is essential (13, 14). Resveratrol was shown to be a scavenger for superoxide radical (O^{2-}), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), nitric oxide (NO) and nitrogen dioxide (NO_2). The main mechanisms of **scavenging free radicals** are the transfer of hydrogen atoms and sequential electron transfer (13).

Reactive oxygen species attack polyunsaturated fatty acids of cell membranes, resulting in lipid peroxidation. Among the main products of lipid peroxidation, malondialdehyde is considered the most mutagenic, while 4-hydroxynonenal is the most toxic (15). Some studies concluded that resveratrol inhibited lipid peroxidation **more efficaciously** than the antioxidative vitamins C and E, which was attributed to its lipophilic and hydrophilic characteristics (13).

Furthermore, resveratrol also acts as a **chelator**. It binds metal ions such as copper and iron ions, and prevents the entering of a metal ion into an oxidative reaction. This consequently prevents the generation of reactive oxygen species and ultimately oxidation (13).

RESVERATROL IN PHOTO-AGEING AND PHOTOCARCINOGENESIS

The most ubiquitous physical carcinogen in our natural environment is **UV radiation**. Depending on the wavelength, there are short-wave UVC rays, mid-wave UVB and long-wave UVA rays. Because UVC rays are blocked by the ozone layer of the Earth's atmosphere, UVB and UVA rays are responsible for inducing skin disorders, including photodamage and skin cancer (16).

Photo-ageing or the disruption of the dermal structure and skin's connective tissue (especially collagen) caused by UV radiation harms many skin functions. Matrix metallopeptidase enzymes (MMPs) are assumed to be responsible for the degradation of collagen and other extracellular matrix proteins. These proteins are also targets for relieving skin photo-ageing. Sirt1 is a putative anti-ageing enzyme that decreases MMP-9 transcriptional expression in the skin. Recent *in vitro* scientific research indicates that Sirt1 could have a therapeutic value, which was also shown on a mice skin model after UV-mediated photo-ageing. Natural Sirt1 agonists, such as resveratrol, may thus have potential as novel therapeutic or cosmetically active ingredients for use in age-related skin conditions, such as photo-ageing (17).

UV radiation may cause mutagenic effects. Initially, it can cause sunburn, which typically increases the risk of skin cancer later in life, particularly in terms of long-term excessive exposure. However, the risk of skin cancer can be increased by years of sun exposure during outdoor activities, even without sunburn. Other factors that increase the **risk of skin cancer** are skin type, family and personal history of skin cancer, frequent use of tanning beds, multiple moles, etc. (7).

Skin cells respond to UVB-induced damage both by tolerating and repairing it via the activation of antioxidants and DNA repair mechanisms or, ultimately, by undergoing programmed cell death when the damage is irreversible. Unfortunately, some damaged cells escape apoptosis, lose mitotic and differentiation control and become cancerous cells (18).

Available experimental data suggest that resveratrol has the potential as a **chemopreventive agent** for UV radiation-mediated skin damage. Numerous studies have investigated its various molecular mechanisms of action (Figure 2). Resveratrol is involved in the regulation of cell cycle phases, cell proliferation, apoptosis, autophagy, tumour promotion and various cancer-related gene expressions, and in the level of production of reactive oxygen species (7).

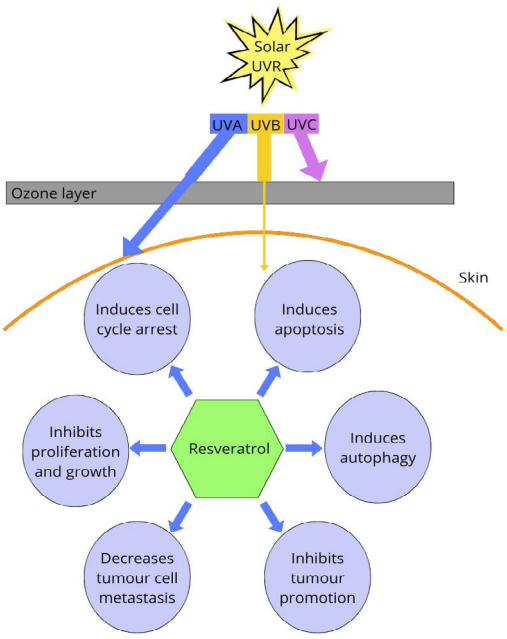

Figure 2: Molecular mechanisms of resveratrol in UV radiation-induced skin cancer; adopted from (7).

SKIN-WHITENING, ANTI-WRINKLE AND ANTI-ACNE BENEFITS OF RESVERATROL

Antioxidants also control oxidative stress correlated with pigmentation disorders, extrinsic skin ageing and inflammation (19).

In vitro and *in vivo* studies have shown that resveratrol may be used as a **whitening ingredient** in cosmetic preparations (3). Dermally applied, *in vivo* investigation with 1% resveratrol dissolved in ethanol and propylene glycol (3:7, V/V) showed a reduction in pigmentation induced by UV radiation in guinea pigs. It decreased cellular melanin synthesis through the inhibition of tyrosinase and inhibition of processes such as tyrosinase gene expression, tyrosinase protein maturation and autophagy (19, 20). It can also be used as an additive compound, especially with a *Morus alba* extract in skin-whitening cosmetics: an *in vitro* study showed a significant synergistic effect. The mixture inhibited 50% of tyrosinase activity, while a single substance (resveratrol or plant extract) inhibited about 25% of activity (21).

Because of its chemical instability, **resveratrol derivatives** have also been evaluated for cosmetic use. *In vitro*, resveratryl triacetate was found to be more resistant to oxidative discolouration and it was less cytotoxic, while a high level of anti-melanogenic activity was retained compared to the effects of resveratrol alone (22). Later, the skin-whitening effects of resveratryl triacetate were examined on 22 human subjects (23). A cosmetic product containing 0.4% of resveratryl acetate or control (i.e. without resveratryl acetate) was applied on one face side twice daily (mornings and evenings). Significant depigmentation occurred in the test group compared with the control group after eight weeks of application.

One scientific *in vivo* study on 42 rats included the application of 50% glycolic acid gel first, followed by the application of a 0.7% resveratrol gel for 15 days. It was concluded that resveratrol increases dermal and epidermal thickness, and has the potential to improve skin with wrinkles (24).

In **acne-prone skin**, resveratrol's antibacterial properties against *Propionibacterium acnes* are believed to make a significant contribution (3, 25). A clinical study with 20 acne vulgaris patients was conducted using a 0.0001% resveratrol gel and a vehicle alone as the placebo (26). A 60-day application on the right side of the face (vehicle on the left side) resulted in a clinically relevant and statistically significant decrease in acne lesions.

SAFETY AND USE OF RESVERATROL IN COSMETICS

In vitro and *in vivo* studies concluded that resveratrol is a **safe and well-tolerated ingredient** for dermal use (3). Ingredient manufacturers recommend 0.1% to 1% of resveratrol in cosmetic formulations (8), while significantly lower (0.0001%) concentrations were also tested in clinical studies (26). The **optimum pH range** is from 3 to 7. Higher pH values or contact with strong oxidising agents may lead to the discolouration of cosmetic products (8).

Cosmetic formulators need to be aware of the resveratrol concentration and vehicles used in products that were tested for safety in scientific research. A case report of a 69-year woman using a cream with resveratrol was reported. Allergic contact dermatitis was confirmed after patch testing with 10% resveratrol in paraffin (27). In another study, dermally used resveratrol and resveratryl triacetate as 0.1 and 0.5% solutions in squalane were used for patch testing (22). Concentrations of 0.1% resveratrol, and 0.1 and 0.5% resveratryl triacetate did not induce any skin reactions in any of the subjects, while 0.5% resveratrol induced slight spotty or diffuse erythema in four subjects. The skin reaction induced by the 0.5% resveratrol solution was classified as **mild irritation**.

RESVERATROL IN MODERN COSMETIC SCIENCE – ADVANCED DELIVERY SYSTEMS

Small hydrophilic polyphenols incorporated into cosmetic products are typically released faster and penetrate into the skin better. In addition, emulsions with a low-lipid content accelerate polyphenol diffusion due to lower viscosity, therefore providing higher release and permeation rates. Those molecules concentrate in the epidermis and dermis, which are the main targets for anti-ageing formulations (1).

In contrast, more hydrophobic molecules, such as resveratrol, are located inside the dispersed lipid phase of oil/water emulsions and they must overcome this interface corresponding to slower diffusion into the skin (1). Because of the poor skin permeation and photodegradation that reduces the activity of resveratrol, it is a major challenge to develop a suitably formulated cosmetic product that facilitates the beneficial effects of resveratrol. As a result, many advanced delivery systems have been created, such as **liquid crystals**, **liposomes**, **nanoparticles**, **nanocapsules**, etc. (5).

CONCLUSION

Based on current evidence, resveratrol acts as a preventive ingredient in many skin conditions, making it a useful compound for cosmetic and dermatological applications. Because of its low water solubility and photo-instability, it is challenging but not discouraging to develop a suitable cosmetic product that facilitates the **beneficial effects of resveratrol as a cosmetically active antioxidant**.

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From farm to skin: Proposal of a simple grape hydro-glycerol extract method for use in natural cosmetics

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ABSTRACT

The increased demand for cosmetics worldwide has raised awareness about safety issues. The concern regarding green chemistry, which is the concept of chemistry acting to protect the environment by developing procedures that limit the use and generation of hazardous substances, has raised the idea of green beauty. In the area of natural cosmetics, there is a need to understand extraction methods using non-toxic solvents of natural origin, such as glycerol and water, and present evidence-based procedures that are helpful for homemade preparations. This study aims to present a process for the simple preparation of a grape hydro-glycerol extract that can be incorporated into a cosmetic product and reproduced in a domestic environment.

Keywords: cosmetics, grapes, hydro-glycerol extract, natural ingredients

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INTRODUCTION

Mirror, mirror on the wall, who's the fairest of them all? **Pursuing a perfect body image** is a global concern (1). Women's preoccupation with fair skin has been widely studied (2). Fair skin serves as symbolic capital and a visual agent in defining boundaries of cultural identity and identifying a person's place in the local social hierarchy, if not the increasingly global hierarchy (3). To achieve such results, people tend to use cosmetics to maintain the skin, and thus the body as a whole, in good condition, to protect it from the effects of the environment and ageing processes, to change its appearance and to make the body smell nicer (4).

The **safety of cosmetics** has always been a top priority on the growing cosmetics market (5). The most common ingredients in cosmetics that have received significant concern include fragrances, preservatives, ultraviolet absorbers and hair dyes (6). New information about the safety of cosmetic ingredients dictates the pace of legislation, and some substances may become prohibited or new substances allowed (7).

These are some of the reasons why the concept of **green beauty** has been gaining interest. It is associated with eco-consciousness, environmental acceptability, sustainability and organic farming. It involves understanding nature and the human body as a whole, improving our looks naturally and holistically, and abstaining from hazardous substances. Green means that a cosmetic product has been formulated without ingredients that are harmful to the skin, body or the environment. It should also contain certified organic ingredients whenever possible, and be packed in recyclable or recycled boxes or bottles.

GRAPES

Grapes (*Vitis vinifera*) are the fruit of the winegrape, a species of the Vitaceae family. It is one of the most consumed fruits globally, primarily in juice and wine, but some grapes are destined for fresh consumption or dried into raisins (8). Archaeological records suggest that cultivation of the domesticated grape, *Vitis vinifera* subsp. *vinifera* began 6,000 to 8,000 years ago in the Near East from its wild progenitor, *Vitis vinifera* subsp. *sylvestris* (9). After pruning in January, clusters form in the spring, and the grapes gain colour, aroma and taste during the summer. The harvest occurs when the grapes are already ripe between September and October, when their weight, colour and acidity present the ideal conditions for wine production (10).

The various components of grapes make them an excellent ingredient to be added to cosmetic formulations. **Resveratrol**'s proven ability to act as an antioxidant on the skin's surface or after penetrating the skin barrier and its anti-ageing activity make it an ideal cosmetically active ingredient for cosmetic formulation. It can also stimulate the proliferation of fibroblasts and increase the concentration of collagen III (11). **Phenolic acids and flavonoids**, such as ferulic acid, caffeic acid, gallic acid and proanthocyanidins, are also efficacious protectors that reduce oxidative stress and may be considered essential in cosmetic formulation for post-sun skin care (12). Grapes also provide other phenolic components, such as anthocyanins, catechin, epicatechin, conjugated flavonoids, and oleic, linoleic and linolenic acids, to counteract the symptoms of epidermal ageing and delay the process of photo-ageing (13, 14).

Scientific evidence has shown that the **antioxidative properties** of polyphenols play a major role in protection against UV radiation following the dermal use of grape extracts (15, 16). In addition, a study performed *in vivo* in *Drosophila melanogaster*, using SMART and Comet assay, showed that grapes have antigenotoxic properties (17, 18). Antigenotoxic properties, in turn, have been linked to anti-ageing properties (19, 20).

GRAPE EXTRACTION

Different studies have been performed to find the best extraction method capable of adequately recovering valuable phenolic compounds. In conventional solid-liquid extraction, bioactive molecules diffuse through the cell wall and cell membrane to reach the extraction solvent. Innovative extraction techniques damage cell walls/membranes and intensify the recovery of phenolic compounds. Despite the high efficiency of these innovative methods, there are still some cost and scalability challenges involved (21).

On the other hand, solid-liquid **extraction using glycerol and water** can be considered a low-cost and efficient way to recover phenolic compounds. It is a beneficial technique for cosmetic applications since the hydro-glycerol extract can be directly integrated into many cosmetic formulations. Thus, the subsequent step of solvent elimination is not necessary. For this reason, such low-cost and easy extraction methods are favoured to obtain safe and high-quality extracts in a domestic environment (22).

This is in line with **green chemistry**, which is the concept of chemistry acting to protect the environment by developing procedures that limit the use and generation of hazardous substances, and the circular economy, the goals of which are resource sustainability, balancing economic growth and environmental protection (22).

GLYCEROL AS AN EXTRACTION SOLVENT

Glycerol, also referred to as glycerine, is a trihydric alcohol with the molecular formula $C_3H_5(OH)_3$. Its IUPAC name is propanol-1,2,3-triol. It is colourless, odourless, has a sweet taste, and is very viscous and hygroscopic. It was discovered in 1789, and was first used in medicine and pharmacy around 1846 (23).

It is a liquid obtained through the alkaline hydrolysis of animal fat or vegetable oils, where the fats, which are glycerides or esters of the fatty acids, are broken up into glycerol and fatty acids (24).

Glycerol has around 2,000 applications and plays a vital role in nature. It is used in foods, pharmaceuticals, personal care products, industrial applications, etc. Newly discovered applications of glycerol have generated a great deal of interest recently due to its expected effects on the market (23).

Glycerol can be used on the epidermis, including mucous membranes, for many cosmetic and medicinal purposes, both external and internal (24). Its main effect is **skin moisturisation**, with essential applications in water-based products such as emulsions, hydrogels and water solutions. Glycerol is typically used in skin and hair care in concentrations from 1 to 5% (25). Undiluted, it is an irritant. It is helpful to keep substances moist due to the tendency to absorb water from the air, which is called humectant activity (24).

Hydro-glycerol systems are being exploited to extract valuable components from plant waste generated in various industrial technologies (26). Indeed, the spectrum of application of glycerol as a solvent has expanded to the extraction of polyphenols (27, 28). Eyiz et al. (13) stated that its mixtures with water, commonly known as **hydro-glycerol or hydro-glycerine extracts or glycerites**, are helpful for obtaining plant extracts that have value due to their content of phytochemical compounds such as phenols, flavonoids, anthocyanins, proanthocyanidins and ascorbic acid.

The harnessing of the properties of glycerol is largely in line with the concept of 'green chemistry', to which the use of the solvent for obtaining natural phytocompounds can be referenced (29–32).

Finally, glycerol is also characterised by its low price, ease of purchase and suitability for consumption purposes (33).

Use of glycerol in solid-liquid extraction of polyphenols

The use of glycerol as an extraction solvent or stabilising agent has been well-documented (34–37). A study showed that using glycerol to extract olive leaf polyphenols is more efficient than using a hydro-alcoholic solution (29).

AIM OF THE STUDY

Based on the aforementioned properties of glycerol as an extraction solvent suitable for extracting **cosmetically active polyphenols from grapes**, this study aims to present a method for the simple preparation of a grape hydro-glycerol extract that can be incorporated into a cosmetic product and reproduced in a domestic environment.

MATERIALS AND METHODS

Chemicals

Glycerol (CAS Number 56-81-5), with a purity of 99.5%, was purchased from PlenaNatura (Amadora, Portugal). Distilled water was purchased from MedicalShop (Ponte de Lima, Portugal).

Grape harvest and preparation

Red Grapes (variety 'Touriga Nacional') were obtained from an organic farmer in September 2022 in the Trás-os-Montes area of Portugal. Before the experiment, whole grapes were air-dried for two weeks away from direct sunshine, at room temperature between 20 and 25 °C. They were then ground using a coffee mill to obtain particles measuring <2 mm, with a paste-like consistency.

Equipment cleaning and disinfection

It was necessary to clean and disinfect the equipment to **minimise the risk of contamination**. For that purpose, a cleaning solution, alcohol (at least 60% alcohol by volume) in a spray bottle, boiled water and clean rags were required.

Protective clothing was used, and hair was tied back. Work surfaces were cleaned with a cleaning solution and sprayed with alcohol. Surfaces were dried with a single-use paper towel. Metal, silicone and glass containers were disinfected and sterilized by boiling them in water for 20 minutes and drying them with a single-use paper towel. Each item was then sprayed with alcohol, ensuring it reached the inside surface of the containers and lids. Items were dried with a single-use paper towel. Tools and non-heat-resistance plastic containers were sprayed with alcohol, ensuring it reached the inside surface of the containers. The containers and tools were air-dried.

Grape hydro-glycerol extract

The weight-to-volume (W/V) method was used. Glycerol acts as a solvent when preparing hydro-glycerol extracts. Plant material can be soaked in a glycerol solution of at least 55% or higher since lower glycerol concentrations are not potent enough to keep mould growth at bay (38, 39). We suggest using 55 to 60% glycerol by volume in the medium for extracting dry plant material. This percentage of glycerol helps to enhance solubility, acts as a stabiliser, facilitates the mixing of ingredients together due to suitable viscosity, helps to maintain moisture content and ensures an adequate preservative action (40).

A 12.5% grape extract in a 60:40 glycerol:water solution was made (or 14.29 g of grapes in 100 mL of total solution, or a DER of 1:6.99 W/V). The exact formulation is as follows, as well as the precise weight of a 200 g batch.

Table 1: Composition of grape hydro-glycerol extract

Ingredient	%	200 g
Glycerol	52.5	105
Distilled Water	35.0	70
Grapes	12.5	25

1. 25 g of dried grapes were weighed in a sterilized jar (Figure 1A).
2. Glycerol and water were pre-mixed in a separate sterilised container (Figure 1B).
3. The medium was added to the jar containing the dried grapes (Figure 1C).
4. The mixture was stirred, ensuring all dried grapes were wet.
5. A square piece of natural waxed paper was placed on top of the jar, and the jar was sealed with the lid (this prevented possible contamination from the chemical coating that may be on the lid).
6. The mixture was shaken daily for two weeks.
7. The preparation was filtered using a coffee filter, and then labelled and capped.

The mixture was stored in an airtight, light-resistant container, and exposure to direct sunlight and excessive heating was avoided.

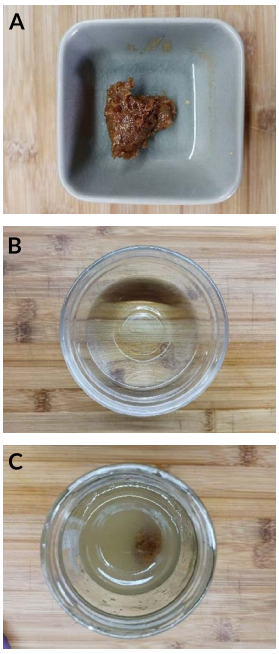

Figure 1: Steps of the preparation: A. Dried and grounded grapes with a paste-like texture; B. Pre-mixture of glycerol and water; and C. Mixture of dried grapes and water and glycerol.

RESULTS AND DISCUSSION

The **pH** of the grape hydro-glycerol extract was determined using pH stripes, and was in the range of 4 to 5. This may have an impact on how much of the extract will be used and how it will be used. It may help reduce the overall pH of the final product. For this reason, we suggest using it at a concentration of between 5 and 10%. The extract can be incorporated into formulations such as emulsions, hydrogels and water solutions.

Since grapes are rich in anthocyanins that are relatively thermolabile (41), the final extract is considered **heat-sensitive**. We therefore suggest adding it in the cool-down phase or when the product is under 70 °C. Using **clean equipment** is good practice for minimising contamination and microbial growth. Because **microbial contamination** is very common in water-containing products, all materials should be clean and sanitised when making hydro-glycerol plant extracts and hydro-glycerol extract-containing formulations. Since the use of kitchen equipment is common when producing homemade cosmetic formulations, we should also stress that the equipment must be used for formulating purposes only.

Demineralised water can be used as an alternative to distilled water. **Distilled and demineralised water** are produced through different purification methods, resulting in a different end product. Demineralised water has had minerals removed so that only H_2O is left. The problem with demineralisation is that it will not remove bacteria or viruses as the process of distilling would. Distillation is a very efficacious method in this respect. It is therefore necessary to boil demineralised water for about 20 minutes to kill the microorganisms.

Stability testing is also essential. The purpose of the stability testing of cosmetic formulations is to ensure that a new or modified product meets the intended physical, chemical and microbiological quality standards, as well as functionality and aesthetics when stored under normal conditions (42). Physical/chemical stability testing includes temperature variations, cycle testing, centrifuge testing, light-exposure testing, mechanical shock testing and the monitoring of organoleptic changes. Microbiological stability testing includes screening and quantitative tests. Packaging stability tests include glass tests, weight loss tests and leakage tests (42).

Stability testing should also be done in a domestic environment, for homemade preparations, to ensure that a product will fulfil its initial function, stay unchanged and remain safe. Although homemade stability tests typically cannot be done with specialised equipment and at a level totally comparable to laboratory conditions, they provide essential results.

For that purpose, we advise **exposing a product to different conditions**. The response to these conditions is examined by measuring or observing one or more parameters (43, 44):

- pH: monitoring the pH of a product at various time points;
- weight: examining changes in weight using a good-quality scale;
- container changes: detecting unwanted interactions between the product's ingredients and packaging;
- viscosity: basic observation in viscosity changes; and
- organoleptic evaluation (e.g. appearance, odour and coloration): changes in smell and appearance typically suggest, for example, oxidation or emulsion instability.

A document containing details of the manufacture of each batch is also required. The **batch manufacturing report** should include raw material, final product and manufacturing process characteristics: batch number, composition, size and weight, storage conditions, the master formula of the batch, start and end process date, product expiration date and, if the purpose is for selling, the manufacturer's license number, etc.

CONCLUSION

With **increased environmental awareness**, there is growing interest in natural ingredients used in cosmetic products. To make natural ingredients an exciting and economically sustainable source of bioactive compounds, selecting and optimising suitable environmentally-friendly extraction technologies that facilitate the extraction of target cosmetically active compounds are indispensable.

The extraction of grapes in a hydro-glycerol mixture is an easy and environmentally-friendly method for obtaining an extract suitable for use in cosmetic formulations, and is **supported by scientific evidence** in terms of cosmetic activity.

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Plantain (*Plantago*) species in skincare

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ABSTRACT

Plantago species are widespread herbs but are currently referred to as weeds. However, *Plantago* extracts and their constituents show promising properties as cosmetic ingredients. Anti-inflammatory, antioxidative and photo-protective activities were shown for aucubin and catalpol, and moisturising properties for polysaccharides. The extract as a whole has antioxidative, antibacterial and anti-inflammatory properties that make it useful for the care of impure skin and acne, or to boost the antimicrobial activity of preservatives. We conclude that *Plantago* species show promising cosmetic potential and should be more widely exploited in the future.

Keywords: extract, *Plantago lanceolata*, *Plantago major*, plantain

Article category: Scientific review article
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ABOUT *Plantago* SPECIES

The genus *Plantago*, which belongs to the *Plantaginaceae* family, comprises about 275 species distributed worldwide (1). The best-known representatives on Slovenian soil are the broad-leaved (*Plantago major*) and the narrow-leaved plantain (*Plantago lanceolata*) (Figure 1).

Plantago lanceolata is a perennial herb that grows 10 to 40 cm high and occurs on lawns, meadows and uncultivated soils. The leaves grow from a ground rosette, are lanceolate and have 3 to 7 parallel veins (2). The flowers are inconspicuous with yellowish stamens that later develop into tiny oval seeds. ***Plantago major*** is also a perennial herb that grows 15 to 30 cm tall and has oval leaves with 5 to 9 parallel veins. The flowers are small and have purple stamens that later develop into very small, oval seeds. Plantain species are wind pollinated and are propagated by seeds. Each plant can produce up to 20,000 seeds (3).

Figure 1: On the left, broad-leaved plantain (Plantago major) and on the right, narrow-leaved plantain (Plantago lanceolata) (photo: Činč Ćurić).

Plantago species have been used in traditional medicine for centuries for their anti-inflammatory, antifungal, analgesic, antitumor, antibacterial and antiviral activities (3–5). In this article, we describe the main active compounds, namely iridoid glycosides aucubin and catalpol; phenylethanoid glycosides verbascoside and plantamajoside, and polysaccharides. All of the mentioned compounds have important biological functions and have come into focus as **natural cosmetic ingredients**. Indeed, verbascoside and different *Plantago* extracts have already been recognised as cosmetic ingredients and are defined in the CosIng database.

IRIDOID GLYCOSIDES – AUCUBIN AND CATALPOL

Details of chemistry

Aucubin is a widespread active ingredient that is difficult to obtain in large quantities because its content in plant material is low, and the compounds are unstable and susceptible to hydrolysis. Chemically, aucubin consists of a cyclopentano-[C]-pyran skeleton (Figure 2A) and has a single glycosidic bond that breaks down to aglycones in acidic environments (6). Aucubin is soluble in water, where it undergoes spontaneous oxidation to form insoluble substances. It is soluble in methanol and ethanol, and insoluble in organic solvents such as ether, chloroform and benzene (7).

Catalpol is a polar molecule that differs from aucubin by the 7,8-epoxy ring on the cyclopentano-[C]-pyran skeleton (Figure 2B). It is soluble in water but unstable at high temperatures (8).

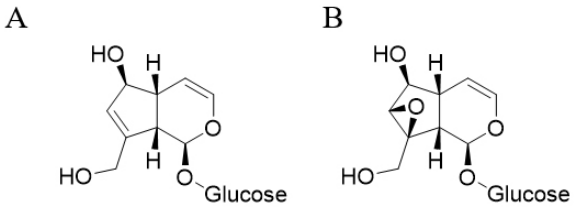

Figure 2: Structural formulas of aucubin (A) and catalpol (B).

Laboratory studies

The active ingredient aucubin has been tested in numerous studies and has shown **anti-inflammatory** activity through various inflammatory pathways (9, 10). It reduces the release of inflammatory mediators such as interleukins and tumour necrosis factor α (TNF-α) (11). Both aucubin and catalpol have been shown to exert **antioxidative effects** by reducing reactive oxygen species (also known as ROS) and monoaldehyde (MDA). Aucubin and catalpol also increased the antioxidative effects of enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase, which can eliminate ROS (12, 13).

Through its anti-inflammatory action, aucubin promoted **wound healing** in the oral mucosa of mice by stimulating early re-epithelialization and collagen matrix formation. The authors concluded that aucubin may be useful for oral wound healing and can be used as a topical agent for oral wounds (14).

Examined cosmetic applications

In a study by Dąbrowska and Nowak, the influence of a cosmetic macroemulsion containing aucubin on skin parameters was tested on 25 female volunteers with an average age of 27 ± 2 years using skin testing devices. Two creams were prepared, one of which contained water, shea butter (5.0 wt.%), monoi oil (5.0 wt.%), cetyl alcohol (6.0 wt.%), glycerylmonostearate (4.0 wt.%) and Microcare® DB (1.0 wt.%). The other cream had the same composition with the addition of aucubin at a concentration of 0.5 wt.%. The skin parameters were measured using the following devices: Tewameter® TM 300 (transepidermal water loss, TEWL), Corneometer® CM 825 (skin hydration), Cutometer® MPA 580 (skin elasticity), Visioscan® VC 98 (skin topography) and Visioline® VL 650 (skin macrorelief). The results showed that the aucubin macroemulsion reduced transepidermal water loss and **increased skin moisture** content compared to a cream without aucubin. The addition of the active ingredient also reduced the value of the total wrinkle area and decreased the average length and depth of individual wrinkles (15).

In the next study, **aucubin and catalpol hydrogels** were prepared. The hydrogel consisted of glycerol (10 wt.%), hydroxyethylcellulose (2.5 wt.%) and Microcare® SB (0.2 wt.%). Hydrogels containing iridoids had the same composition with the addition of 0.5 wt.% of aucubin or catalpol. Other hydrogels were prepared in which the iridoids were loaded into lipid nanoparticles. The same tests were performed as described above. A synergistic effect was observed between the iridoids and the hydrogels on moisturising and anti-ageing effects. The hydrogel without iridoids increased epidermal moisture content by 12% and decreased transepidermal water loss by 25%. Hydrogels with aucubin and catalpol increased epidermal moisture content by 42% and 49%, respectively. Total transepidermal water loss was reduced by 47% and 35% for aucubin and catalpol, respectively. Hydrogel containing aucubin encapsulated in lipid nanoparticles increased epidermal moisture content by 46% and decreased transepidermal water loss by 52%, while hydrogel with catalpol encapsulated in lipid nanoparticles increased epidermal moisture content by 60% and decreased water loss by 49%. Cosmetics with the active ingredient aucubin caused a reduction in the values of all skin macrorelief parameters compared with the other tested hydrogel formulations, namely by 10% in the total wrinkle area and by 6% and 4% in the average length and depth of wrinkles, respectively (16).

The next application of aucubin was its ability to attenuate **photo-ageing**. Photo-ageing is a common cause of skin deterioration in which oxidative stress from UV radiation upregulates enzymes responsible for collagen damage in the skin. In a study by Ho et al., aucubin inhibited the production of these enzymes by 57%. It inhibited the formation of ROS and MDA levels, and it increased glutathione levels. The authors concluded that aucubin may play an important role against UV-induced photo-ageing (17).

PHENYLETHANOID GLYCOSIDES – VERBASCOSIDE AND PLANTAMAJOSIDE

Verbascoside: Details of chemistry

One of the representatives of phenylethanoid glycosides is the compound verbascoside (Figure 3A). **Verbascoside** is chemically composed of caffeic acid (phenylpropanoid moiety) and hydroxytyrosol (phenylethanoid moiety) bound to the sugar α-L-rhamnopyranosyl-β-D-glucopyranose via ester and ether linkages (18).

Laboratory studies

Traditionally, plants containing high amounts of verbascoside have been used to treat i**nflammation and microbial infections**. Verbascoside exhibits anti-inflammatory activity by reducing the release of proinflammatory cytokines (19).

Studies have also shown that verbascoside promotes skin repair and relieves skin inflammation. These properties are attributed to its antioxidative and ROS scavenging effects. Because of its anti-inflammatory properties, verbascoside has been studied for the treatment of atopic dermatitis. An *in vivo* study was conducted on mice, and the results showed that verbascoside revealed the symptoms of atopic dermatitis such as scratching and severity of skin lesions, which makes it a potential therapeutic agent for the treatment of atopic dermatitis (20).

The same conclusion was drawn in the study by Biasibetti et al., in which a combination of a topical lactoferricin/verbascoside emulsion was prepared and tested in dogs with atopic dermatitis. Topical emulsion was administered daily for two weeks to 38 dogs. No adverse effects were reported, and a reduction in bacterial overgrowth and clinical signs in the skin folds of atopic dermatitis was observed. However, it is important to note that the study did not include a control group (21).

In an *in vitro* study by Kostysuk et al., verbascoside showed **promising antiphoto-ageing activity** on keratinocytes, and exhibited high photo-stability and low photo-toxicity. Verbascoside was able to absorb UVA and UVB radiation, reduce UV-induced overproduction of free radicals and downregulate the expression of inflammatory cytokines (22).

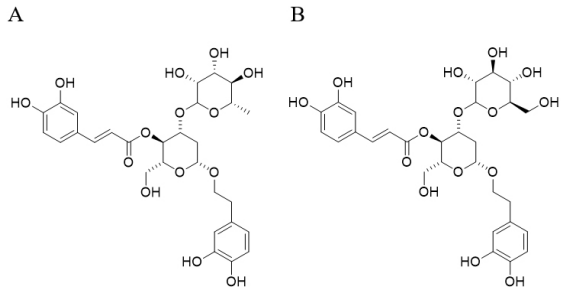

Figure 3: Structural formulas of verbascoside (A) and plantamajoside (B).

Plantamajoside: Details of chemistry

Plantamajoside is another representative of phenylethanoid glycosides that differs structurally from verbascoside in terms of sugar moiety (glucopyranosyl instead of rhamnopyranosyl) (Figure 3B) (23).

Laboratory studies

Plantamajoside exhibits **anti-inflammatory activity** and has inhibitory effects on 5-lipoxygenase and cAMP phosphodiesterase (24). In a review by Ravn and Brimer et al., plantamajoside was also shown to possess antimicrobial activity (25).

Like verbascoside, it is a potential candidate for **inhibiting photo-ageing** of the skin. This was investigated in a study by Han A.R., M.H. Nam and K.W. Lee, in which plantamajoside attenuated the expression of enzymes involved in melanin synthesis. The study was conducted *in vitro* using human cell lines of keratinocytes and dermal fibroblasts. In addition, the authors found that plantamajoside attenuated the expression of inflammatory cytokines (26).

POLYSACCHARIDES

Other very important compounds isolated from *Plantago* species are **polysaccharides** found in the leaves or seeds. They differ in terms of molecular structure and molecular weight. The polysaccharides isolated from the leaves have a pectin-like structure with galactose, rhamnose, and galacturonic acid as predominant monosaccharides. As reported by Kardošova et al., the polysaccharides had a pectin-like structure and were classified as rhamnogalacturonan, arabino-galactan and α-D-glucan (27). Samuelsen reported the isolation of an acidic arabinogalactan and a highly esterified pectic polysaccharide (28).

The polysaccharides from the seeds have an arabinoxylan structure, with arabinose and xylose being the predominant monosaccharides (3).

It is well established that polysaccharides play an important role in various biological functions. Mucopolysaccharides isolated from the seeds of *Plantago* species are suitable for the treatment of **dry and sensitive skin** and have been shown to be important compounds in wound healing and could also limit scar formation (29).

Possible explanations for their **beneficial effects on wounds** are:

- They retain proteins on the wound surface;
- They act as a physical barrier to prevent water evaporation, thus preventing access by micro-organisms; and
- They activate growth factors that accelerate fibroblast function and collagen production, and facilitate the formation of granulation tissue, leading to increased blood supply to the injured tissue and improved wound healing (30).

USE OF WHOLE EXTRACT IN COSMETICS

Although single compounds found in *Plantago* species provide promising effects in skin care, the effect is frequently not due to one compound, but is the **synergistic effect** of the whole orchestra of molecules present in the extract. In the following we will describe the use of *Plantago* extracts as a whole, not just single compounds found in the extract.

Antibacterial action

In a review by Samuelsen, the **antibacterial and antifungal activity** of *Plantago major* water, methanol, and 50% and 70% ethanol extracts were compared in different studies. The activity of the extracts was compared with gentamicin, an antibiotic, as a positive control. Gentamicin had an inhibition zone of > 25 mm, while the methanol extract had a 10-15 mm inhibition zone for *S. typhimurium*. The 50% ethanol extracts were active against the microorganisms *S. aureus*, *B. subtilis*, *S. dysenteriae* and *E. coli*, while the 70% ethanol extracts were most effective against *S. flexneri* and had weaker activity against *S. aureus*, *S. sonnei*, *E. coli*, *Esherichia* 'crim' and *M. smegmatis*. The antifungal activity was also tested in comparison with the antifungal agent nystatin, but the extracts showed weak antifungal activity. The good antibacterial properties are due to the presence of iridoid glycosides, especially aucubin (3, 31–33).

This makes *Plantago* species suitable for incorporation into skin products to **enhance the microbiological protection** of preservatives, and for the treatment of **impure skin and acne** (2).

Skin-whitening action

Melanin is a skin pigment that protects the skin from UV radiation. It is produced by melanocytes in the basal membrane through a process known as melanogenesis. Melanin is formed from tyrosine by an enzyme called tyrosinase. Cosmetic agents used for skin whitening can act in two ways: by inhibiting melanin production or by inhibiting tyrosinase activity (2).

In a study by Yoon et al., *Plantago asiatica* extract inhibited thyrosinase activity by 19.9% at a concentration of 100 μg/mL (when L-DOPA was used as an active substrate), suggesting that *Plantago* extract can be used as a **natural whitener** in cosmetics (34).

Wound healing

Wound healing is a very complex and dynamic process that occurs in a timely and orderly manner. It consists of four phases that may overlap. The phases are hemostasis, inflammation, proliferation and remodelation (35).

Cosmetic products are usually not used for wound healing, but the application of creams or ointments has a **positive effect on skin regeneration and wound care**, where the wound is clear of debris or infection and optimal moisture is maintained (36). Some cosmetic products exert their activities via their anti-inflammatory activity and affect the inflammation phase (37), while others affect the proliferation phase in which fibroblasts play the most important role. In the remodelation phase, the addition of creams can help wound regeneration, leading to reduced scar formation (38).

In a study by Fakhrudin et al., the extract of *Plantago lanceolata* (extract fraction insoluble in n-hexane) showed anti-inflammatory activity. The extract inhibited the expression of the proinflammatory enzymes COX -1 and COX-2, and decreased the volume of paw edema in carrageenan-induced mice, which is a common method for acute anti-inflammatory evaluation. The extract also inhibited leukocyte migration by reducing the level of chemokines (39).

In a study by Ashkani-Esfahani et al., 36 male Sprague-Dawley rats were randomly divided into three groups. The first group received no treatment, the second group received the gel treatment and the third group received a mixture of 5% *Plantago major* and *5%* aloe vera gel treatment. The group with the mixture of *Plantago major* and aloe vera demonstrated a faster wound closure rate than the first and second groups. It was also observed that the numerical density of fibroblasts, the volume density of collagen bundles, the mean diameter and the volume density of the vascular group were significantly higher than those of the control group and the group treated with gel, which means that the mixture promotes fibroblast proliferation, re-vascularization and the synthesis of collagen bundles (40).

Another study was performed on 72 mice divided into four groups. The first group received an ointment containing 10% *Plantago lanceolata* extract, while the second group received an ointment containing 20% *Plantago lanceolata* extract. The third group received vaseline and the fourth group received no treatment. The wounds were then examined on days 7, 14 and 21, and the authors concluded that epithelialization was more pronounced in the second group, while vascularization and collagen deposition were more advanced in the first and second groups than in the other groups, making *Plantago lanceolata* extract a potential candidate for wound healing (41).

Clinical study on burns – a case control study

A study was conducted on 15 patients with second-degree burns, aged between 21 and 49 and with a body mass index between 18 and 25, who had suffered a burn with a flame or hot liquid in the previous 24 hours. One side of the burn was treated as the intervention group and the other side as the control group. The control group received 1% silver sulfadiazine ointment, while the intervention group received *Plantago major* ointment (10% *Plantago major* extract in Vaseline). The ointment was applied to a sterile gauze and the wound was bandaged with these gases. The mean recovery time was not statistically significantly different between groups (control 13±2.65 and intervention 11.73±2.22 days), indicating that the *Plantago major* ointments had the same effectiveness as the silver sulfadiazine. In addition, infection was measured on day 3 and day 10, and the results were the same in both groups (day 3: 10 positive and five negative for both groups, and on day 10: zero positive and 15 negative for both groups). The patient's pain score was evaluated using the visual analogue score (VAS), in which the patient rated his pain on a scale of 1 to 10 (10 representing the highest and 1 the lowest pain). The scores were evaluated on day 1, day 3 and day 7, and the scores were the same in both groups (42).

CONCLUSION

Plantago species are widespread herbs found all over the world and are currently referred to as weeds. Nevertheless, *Plantago* extracts, and their constituents show **promising properties as cosmetic ingredients** and their properties should be more widely exploited in the future.

The **isolated compounds** exhibited anti-inflammatory, antioxidative and photo-protective properties. Aucubin and catalpol incorporated into creams or hydrogels increase skin moisture content and reduce

transepidermal water loss. In addition, they have a positive effect on wrinkle length and depth. Mucopolysaccharides isolated from the seed have a moisturising effect and can be used to treat dry and sensitive skin. In addition, mucopolysaccharides can limit scarring.

The **extract as a whole** can be used in wound healing by inhibiting inflammation and promoting collagen formation. The extract has antioxidative, antibacterial and anti-inflammatory properties that make it useful for the care of impure skin and acne, or to boost the antimicrobial activity of preservatives.

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With active cosmetics toward better skin health: Natural ingredients with anti-inflammatory activity

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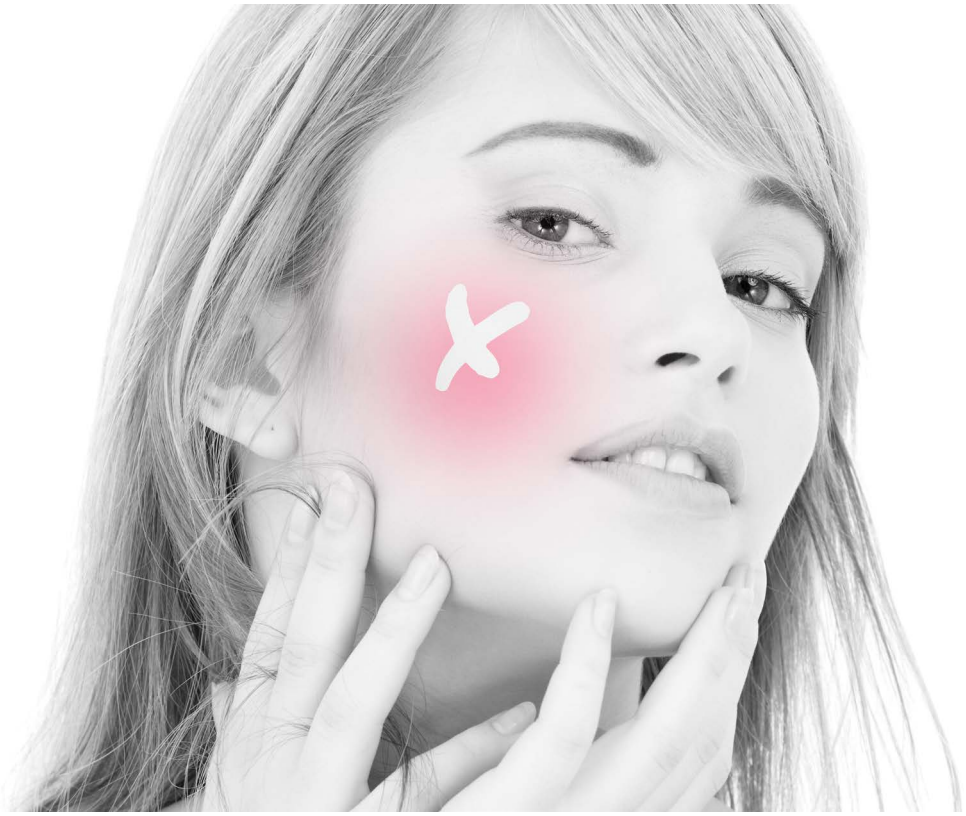
ABSTRACT

Cosmetic products with revitalising properties are the main element of the skincare regime of many women and men as well. Skin renewal is a complex process that involves deep layers and various molecular mechanisms. It is therefore difficult to find a suitable skincare product that will actively contribute to faster skin revitalisation. A healthy immune system and the balance between pro- and anti-inflammatory mediators are crucial to achieving and maintaining good skin condition. This article focuses on natural anti-inflammatory cosmetic ingredients and their positive impacts on the skin.

Keywords: anti-inflammatory, inflammation, natural ingredients, skin renewal



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INFLAMMATION

Skin inflammation develops as a result of allergic and autoimmune reactions or due to chemical and mechanical skin injuries, burns, sunburn and microorganisms. Different **immune cells** are involved in this process and they regulate inflammation by releasing various substances. Among the most well-known are cytokines, which are peptide molecules that stimulate or inhibit inflammation. If the balance between pro- and anti-inflammatory activities is not achieved, i.e. inflammatory factors prevail, chronic or systemic diseases such as atopic dermatitis will develop. Erythema, oedema, burning sensation, itching and pain are the prime **visual signs** of inflammation (1).

In order to **correct the imbalance** in the organism, anti-inflammatory ingredients play an important role. Some anti-inflammatory cosmetic ingredients not only have anti-inflammatory properties but also antimicrobial (e.g. α-bisabolol), antioxidative (e.g. asiaticoside), skin conditioning (e.g. madecassoside), etc. They are therefore beneficial in the **active care** of skin conditions such as acne, atopic dermatitis, psoriasis, burns, wounds, rosacea and aged skin (1).

Some of these cosmetically active ingredients are the focus of this article. Their natural sources and basic chemical characteristics are presented, together with findings of **scientific studies** about their skin-related activities relevant to cosmetic use. In addition, **cosmetic raw materials** available on the market are briefly described. Data were obtained from the manufacturer's documents published on the UL Prospector website (https://www.ulprospector.com/en/na/PersonalCare); see the referenced citations for detailed information.

CENTELLA EXTRACT

INCI name: *Centella Asiatica Extract*
CosIng functions: cleansing, skin conditioning, smoothing, soothing and tonic

Centella asiatica, better known as gotu kola or simply **centella**, is a medicinal plant that has been used in traditional and conventional medicines for hundreds of years. The main active compounds are saponins, chemically called pentacyclic triterpenes: primarily asiaticoside, madecassoside, and asiatic and madecassic acids (2).

On the cosmetic market, asiaticoside and madecassoside are available as **pure compounds**, and also as constituents of **centella extract**. The extract has been shown to be effective in the healing of small and hypertrophic wounds, burns, psoriasis and scleroderma. Studies indicate that it can be used in photo-aged skin, cellulite and stretch marks. As an antioxidant with an antibacterial effect, it reduces acne and promotes tissue regeneration. At the molecular level, fibroblast proliferation, the synthesis of collagen and intracellular fibronectin are increased. Centella extract also has a positive impact on the improvement of tensile strength of newly formed skin and the improvement of hypertrophic scars and keloids (2–4).

Cosmetic application

Centella extract appears as a light-brown to yellow homogeneous translucent **liquid** with a characteristic odour (5), or as a milky white **powder** (6). Since the powder is **not soluble in water** (but soluble in alcohols such as ethanol, propylene glycol and butylene glycol; please note that some of the solvents are not allowed in natural cosmetics certifications), it is recommended to use it in a pre-dissolved form. The ingredient is **temperature resistant**. The recommended cosmetic concentration of powder and liquid form is 0.1 to 2.0% (6) and 1 to 5% (5), respectively.

Preparations of centella have shown a positive impact in the treatment of skin diseases such as acne (an *in vitro* study showed antibacterial activity against *Cutibacterium acnes*), burns (*in vitro* and *in vivo* human studies), atopic dermatitis (*in vivo* animal studies), skin wounds (*in vitro* and *in vivo* animal studies) and alopecia (*in vitro* study) (7).

ASIATICOSIDE

INCI name: *Asiaticoside*
CosIng functions: antioxidant, perfuming and skin conditioning

Asiaticoside induces the synthesis of collagen type 1 in human dermal fibroblast cells. The process is maintained by activation of the TβRI kinase-independent Smad pathway (8). Furthermore, an *in vitro* study performed on fibroblasts of normal human skin concluded that the stimulation of cells with asiaticoside enhanced the expression of genes important for wound healing. Fibroblast proliferation and extracellular matrix synthesis were increased (9).

In vivo animal studies concluded the beneficial healing effects of asiaticoside in wounds (10), burns (11), hypertrophic scars and keloids (12).

Cosmetic application

Due to its sugar unit in the chemical structure, the penetration of asiaticoside into the skin is difficult. Therefore, asiaticoside was incorporated into **liposomes** (13). Other cosmetic suppliers offer asiaticoside in the form of a fine, white and odourless **powder**. The range of concentrations used in cosmetic formulations is 0.1 to 0.5%. Asiaticoside is **soluble in water**, alcohol, propylene glycol, ethoxydiglycol, alcohol 50% (V/V), glycerol, butylene glycol, polyethylene glycol 400 and polyethylene glycol 600. Please note that some of these solvents are not allowed in natural cosmetics certifications. During the formulation of products such as gels and emulsions, asiaticoside should be added to the hydrophilic phase before emulsification with the lipophilic phase (14).

This ingredient can be used in skin and lip care products, and in anti-wrinkle, anti-cellulite and skin repairing products (14).

MADECASSOSIDE

INCI name: *Madecassoside*
CosIng functions: antioxidant and skin conditioning

Madecassoside has proven effects in scar management and wound healing. Skin penetration is limited because of its high hydrophilicity. Since liposomes have a structural similarity to and are biocompatible with cell membranes, they can be used as a delivery system for this ingredient, too (15). An *in vitro* study has proven the antioxidative properties of madecassoside through the protection of endothelial cells against lipid peroxidation and apoptosis caused by hydrogen peroxide (16).

Cosmetic application

Madecassoside is used as a white **powder** in formulations between 0.04 to 1.0%. Since it is **water-soluble**, it can be directly added to the water phase (17).

Madecassoside showed moisturising and anti-inflammatory activities on *Cutibacterium acnes*-stimulated THP-1 human monocytic cell line in one study, suggesting the possibility of using this ingredient in acne-prone skin (18). In addition, an *in vivo* animal study has presented healing properties in burn wounds (11) and a promising treatment option for vitiligo caused by oxidative stress (*in vitro* study) (7).

AZULENES

INCI name: *Guaiazulene*
CosIng functions: antimicrobial and perfuming
INCI name: *Chamazulene*
CosIng function: skin conditioning

Azulenes are a class of compounds with anti-inflammatory and antimicrobial properties. Two well-known representatives are guaiazulene and chamazulene, which are characterised by their intense dark-blue colour. Natural sources are chamomile (chamazulene), cypress pine (guaiazulene) and yarrow (chamazulene) essential oils. Isolated chamazulene is rarely used due to its high cost and is usually replaced by semi-synthetic guaiazulene. Azulene and guaiazulene form solid **crystals**, while chamazulene is a **liquid**. All the compounds are practically **insoluble in water** and have a specific odour similar to naphthalene (1).

In 2003, research by Wang et al. showed the photo-mutagenic effects of azulene and guaiazulene in *Salmonella typhimurium* following irradiation with UVA and/or visible light. The authors concluded that caution should be taken in terms of their cosmetic use. Therefore, the use of essential oils **not isolated compounds** in formulations is recommended (19).

Cosmetic application

Guaiazulene is an FDA-approved cosmetic colour additive. It cannot be added to products made for the around-eye area (20). Chamazulene is not reported to be a common ingredient in cosmetics.

α-BISABOLOL

INCI name: *Bisabolol*
CosIng functions: masking, skin conditioning and soothing

α-bisabolol is a monocyclic sesquiterpene alcohol mainly found in natural sources such as essential oils of candeia (*Eremanthus erythropappus*; up to 85%), chamomile (*Matricaria chamomilla*; up to 50%), Panama plinia (*Plinia cerrocampanensis*; up to 45%) and salie (*Salvia runcinata*; up to 90%). α-bisabolol is mainly produced by semi-synthesis or synthesis, but it can also be isolated from the aforementioned essential oils (1).

The substance is found in nature in the (−)-α-bisabolol form and has been shown to be safe, while the synthetic version, which is a **racemic mixture** that also contains the (+)-α-bisabolol, is still understudied (21). *In vitro* laboratory research has shown that it acts as a skin penetration enhancer and against the herpes simplex virus, and inhibits the synthesis of melanin. Due to its skin-soothing, anti-inflammatory and antimicrobial properties, there is great interest in its use in cosmetics, especially in baby care products (1, 22). In addition, it is able to prevent irritation caused by other ingredients present in a formulation (21).

Although this ingredient is considered safe for use in cosmetics, it can induce allergic reactions in some individuals (23).

Cosmetic application

It is a colourless to pale-yellowish viscous **liquid** with a floral scent (1). It is soluble in **lipids** such as vegetable oils and **alcohol** (ethanol). The ingredient purity of (−)-α-bisabolol is high, typically 85 to 99%. The concentration range used in formulations is 0.07 to 1% for deodorants and skincare products, while in lipsticks the concentration is up to 0.001%. It is intended for any skin type, including the most sensitive, and is widely found in cosmetic products for hair, face and body (1, 21). Dried flowers of chamomile in glycerol are also a source of α-bisabolol (24).

Scientific literature based on *in vivo* animal studies confirmed the healing properties of chamomile extracts on wounds (25), burns (26) and ulcers (also with an *in vitro* study) (27). Furthermore, the clinical evaluation of an innovative ozonated sunflower oil and α-bisabolol spray for the dermal treatment of chronic venous leg ulcers was significantly positive (28).

BOSWELLIC ACIDS

INCI name: *Acetyl Beta-Boswellic Acid*
CosIng function: skin conditioning

The gum-oleoresin of boswellia (*Boswellia serrata*), also known as Indian frankincense, is composed of **boswellic acids** (up to 30%). Chemically speaking, they are pentacyclic triterpenes, and are present as a mixture of α-boswellic, β-boswellic and 11-keto-β-boswellic acids (1, 29).

The mechanism of action of boswellic acids is the inhibition of two pro-inflammatory enzymes, 5-lipoxygenase and elastase, thereby supporting skin texture and integrity. It results in the reduction of inflammation which significantly contributes to the slowing of the skin ageing process (30).

Studies conducted *in vitro* and *in vivo* on animals have shown the benefits of boswellic acids in psoriasis (31), the skin-ageing process (α-boswellic acid acetate) (32) and the UVA radiation-induced photo-ageing of the skin (33). Moreover, the results of an *in vivo* study demonstrated the potential of 3-O-acetyl-11-keto-boswellic acid to protect skin cells from UVA-induced damage by modulating inflammatory mediators and/or the production of free radicals (34). The most common consequence of breast carcinoma treated with radiotherapy is radiodermatitis. An *in vivo* human study has presented the effectiveness of a boswellia-based cream used topically during radiotherapy. The cream was well tolerated by the patients and it reduced the use of topical corticosteroids, the degree of erythema and superficial skin symptoms (35).

In vitro cytotoxicity tests were performed on human skin-derived cell lines and sensitivity was measured. It was concluded that the gum-oleoresin and 3-O-acetyl-11-keto-boswellic acid exert moderate to low skin toxicity (36).

***Boswellia serrata* resin extract**

INCI name: *Boswellia Serrata Resin Extract*
CosIng functions: smoothing and tonic

Cosmetic application

In the cosmetics industry, *Boswellia serrata* extract is usually available as a white to creamish-white **powder** with a characteristic odour. It is **insoluble in water**, but **soluble in alcohol** (37). The recommended concentration of the ingredients from reference (37) is 3 to 5%. It can be used as a fixative in perfumes and as an ingredient for soaps, creams, lotions, gels and detergents (30).

The extract has the potential to act against acne-causing bacteria, and to decrease skin inflammation and wound infections (1).

BETULINIC ACID

INCI name: *Betulinic Acid*
CosIng function: skin conditioning

Betulinic acid is found in a large number of plants and some natural sources include ash (*Fraxinus excelsior*), birch (*Betula pendula*, *Betula pubescens* syn. *Betula alba*) and centella (*Centella asiatica*). A high concentration is found in birch bark: one gram of birch bark contains approximately 10 mg of betulinic acid. Anti-inflammatory properties are represented in its activity as a prostaglandin antagonist. Since it stimulates the synthesis of collagen, it has a positive effect on cellulite, photo-aged skin, wrinkles and burns (1).

It has been scientifically shown in an *in vivo* animal study that betulinic acid is able to attenuate inflammation in psoriasis (38). In addition, as an antitumour agent for cutaneous and subcutaneous tumours, the penetration of betulinic acid-loaded ointment was enhanced with electrochemotherapy (an *in vivo* animal study) (39).

***Betula Alba* extract**

INCI name: *Betula Alba Leaf Extract*
CosIng functions: astringent, cleansing, fragrance, skin conditioning, soothing and tonic

Cosmetic application

Betula alba leaf extract is a yellow to brownish **liquid soluble in water and alcohol**. The pH is 4.5 to 6.5. The ingredient acts as an astringent, hair tonic and antiperspirant, and is helpful with wound healing. The recommended concentration is between 1 to 10% (40).

CAFFEIC ACID

INCI name: *Caffeic Acid*
CosIng functions: antioxidant and masking

Caffeic acid is a natural phenolic compound found in many sources of plant origin such as artichoke (*Cynara scolymus*), lemon balm (*Melissa officinalis*), sage (*Salvia officinalis*), coffee (*Coffea arabica*, *Coffea canephora*) (1). Caffeic acid can prevent melanin production in the skin. In addition, it is an antioxidant that can inhibit the formation of lipid peroxides (41). Due to its antioxidative activity, it is used for ultraviolet radiation-damaged skin and premature ageing. The antimicrobial activity of caffeic acid can be useful to boost the activity of preservatives and is beneficial for products for acne-prone skin. Those two features, the antioxidative and antimicrobial effects, are higher in products with an acidic pH (1).

Cosmetic application

Caffeic acid is a yellowish crystalline **powder**, **poorly soluble in water**, and is used in skincare and skin-lightening products at concentrations of 0.05 to 0.25% and 0.5 to 2.0%, respectively (41).

Scientific data has presented the impact of caffeic acid in many different skin conditions and disorders, such as ageing (*in vivo* and *in vitro* study with artichoke ethanolic extract) (42), UVB protection (*in vitro* and *in vivo* animal study with caffeic acid) (43) and photo-ageing (*in vitro* study with *Coffea arabica* extract and its constituents) (44), aggressive cancers (*in vitro* study with caffeic acid) (45), wound healing (*in vivo* animal study with caffeic acid phenethyl ester) (46), acne and folliculitis (*in vitro* study with caffeic acid) (47) and psoriasis (*in vivo* animal study conducted with extracts of *Melissa officinalis*) (48).

DARUTOSIDE

INCI name: *Darutoside*
CosIng function: skin conditioning

Natural sources of darutoside are St. Paul's wort species (*Sigesbeckia* sp.): *S. glabrescens*, *S. orientalis* and *S. pubescens*. It has many applications: it helps in wound healing, wrinkle treatment and the removal of skin pigmentation spots, and reduces the appearance of stretch marks and skin inflammatory conditions (1).

Cosmetic application

Darutoside is a white crystalline **powder** that is practically insoluble in water (1).

GLYCYRRHETINIC ACID

INCI name: *Glycyrrhetinic Acid*
CosIng function: skin conditioning

Glycyrrhetinic acid, the hydrolytic product of glycyrrhizic acid, together with its salts and esters, is used as a cosmetic ingredient due to its function as a skin-conditioning agent. Glycyrrhetinic acid is a pentacyclic triterpenoid found in liquorice (*Glycyrrhiza glabra*) roots (49). Glycyrrhetinic acid demonstrates antimicrobial, enzyme inhibitory, anti-inflammatory, antioxidant, analgesic and antiviral effects (50).

Skincare products containing glycyrrhetinic acid have been shown to have a positive effect on skin with moderate inflammation, e.g. eczema, burns, diaper rash, facial seborrheic dermatitis and genital itching. They relive the unpleasant feeling after insect stings, and suppress inflammation of the gingival tissue,

mouth, lips and throat (1). Some of these biological properties are important in the treatment of various dermatological disorders in humans. The key active ingredient obtained from liquorice root is 18β-glycyrrhetinic acid (24). It effectively inhibits the activation of tyrosinase, prevents the production of melanin and expresses a whitening effect (51).

Cosmetic application

18β-glycyrrhetinic acid appears as a white and crystalline **powder** insoluble in water, paraffin and vegetable oils. It is soluble in **ethanol** and **1,3-propylene glycol**. The concentration used in cosmetic products is 0.5 to 2% (50, 52). It is stored at temperatures below 20 °C, without direct exposure to UV light (52). It is found in skin care products, toothpastes and mouthwashes. It does not typically cause skin irritation or allergic reactions (1).

The *in vitro* findings demonstrate that 18β-glycyrrhetinic acid exhibits anti-inflammatory action against radiation-induced skin damage, e.g. ionizing radiation (51). Furthermore, scientific articles have shown a positive impact on skin disorders: hyperpigmentation (*in vivo* human study) (53), atopic dermatitis (*in vivo* animal study) (54) and photo-ageing (*in vivo* animal study) (55).

OLEANOLIC AND URSOLIC ACID

INCI name: *Oleanolic Acid*
CosIng function: skin conditioning
INCI name: *Ursolic Acid*
CosIng functions: masking, perfuming and skin conditioning

Oleanolic and ursolic acids are triterpenoid compounds. Natural sources include apples (*Malus pumila*), olives (*Olea europaea*), pomegranate (*Punica granatum*), rosemary (*Rosmarinus officinalis*), etc. Oleanolic and ursolic acids have shown anti-inflammatory effects after dermal application in concentrations of at least 0.2 mg/cm^2 of skin. Ursolic acid has shown a higher cytotoxic activity against human fibroblast cells than oleanolic acid. Ursolic acid accelerates wound healing, as it inhibits pro-inflammatory enzymes, and accelerates the formation of the extracellular matrix (1, 56). Furthermore, ursolic acid was used to prevent pigmentation induced by UV irradiation due to the inhibition of tyrosinase activity, in skincare emulsions for wrinkle treatment, in moisturisation and anti-ageing hydrogels and for lowering transepidermal water loss in formulations for skin hydration (57). The safety and efficacy of oleanolic and ursolic acids after application on the skin have been confirmed in *in vivo* laboratory experiments (1).

Cosmetic application

Oleanolic and ursolic acids come in the form of crystals. Both substances are **insoluble in water** but **soluble in alkaline solutions** (1). As a cosmetic ingredient available on the market, 80% oleanolic acid is a cream **powder** with a green tinge and characteristic odour. It is soluble in methanol (57). 60% ursolic acid comes as a white to cream **powder** with a typical odour. The range of recommended concentrations is 0.5 to 2% in facial skin care products and lip balms (58).

Oleanolic and ursolic acids are used as cosmetically active ingredients in a wide range of cosmetic products. They have been incorporated into facial creams and gels with an anti-wrinkle and soothing action, lip care cosmetics, products for improving and preventing skin roughness, acne and scars, products with a skin-lightening activity and products for regulating hair growth. They are also suitable for skin cleansing preparations (56).

CONCLUSIONS

This article presents the potential of natural substances with anti-inflammatory activity in skin care products. Not only do they possess the covered feature, but also other properties such as antioxidative, antimicrobial, skin-soothing and skin-whitening effects. Due to the growing interest of cosmetic scientists to enrich their formulations with such high-value ingredients, their application will increase even more in the future.

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Hyaluronic acid of different molecular weights: Where size really matters

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ABSTRACT

Hyaluronic acid is a widely used cosmetic ingredient with a good safety profile. Hyaluronic acid of different molecular weights exhibits different effects when applied to the human skin and to different cell types *in vitro*. Lower molecular-weight hyaluronic acid shows promising effects in wound healing due to the activation of immune response and inflammation. Future research will show the complete range of related effects, both positive and negative. On the other hand, high molecular weight hyaluronic acid offers excellent surface moisturising properties and has been deservedly a staple in anti-ageing cosmetic products for years.

Keywords: hyaluronic acid, inflammation, molecular weight, skin

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INTRODUCTION

Hyaluronic acid is considered to be one of the most widely used polymers in the cosmetics industry. It has excellent **humectant** properties, which allow it to bind a large number of water molecules in a meshwork structure. In addition to cosmetics, hyaluronic acid is a highly desired substance for use in a variety of food and medicinal applications, as it is biocompatible, does not induce immune responses and has a high capacity for water retention (1–5).

Hyaluronic acid plays an important part in life processes. It is found in a wide variety of tissues and the organs of animals, including humans, as well as in the capsules of some types of bacteria (5). In humans, the greatest amount is present in the skin, where it is found near **collagen and elastin** fibres, holding them in the proper configuration. In ageing skin, these hyaluronic acid links are gone, which contributes to collagen and elastin fibre disorganisation, fine lines, wrinkles and nasolabial folds (3, 4).

DETAILS OF STRUCTURE

Hyaluronic acid is a widely available polymer that belongs to the glycosaminoglycan family. It comprises repeating units of disaccharides, with molecules of D-glucuronic acid and N-acetylglucosamine molecules linked by β-(1→4) and β-(1→3) glycosidic bond as shown in Figure 1 (4).

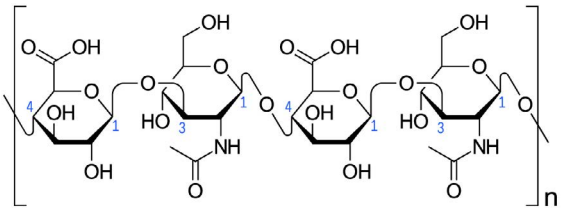

Figure 1: Chemical structure of hyaluronic acid.

Hyaluronic acid is a mild acid. It is widely present in the body in its salt form, sodium hyaluronate (or simply hyaluronate). Hyaluronic acid is also known as **hyaluronan**, which is a general name for all types of hyaluronic acid (1).

In the body, under physiological conditions, hyaluronic acid exists as a **polymer of high molecular weight** (usually with a molecular weight of between 10^3 and 10^4 kDa). However, greater size polydispersity occurs in inflammatory conditions where low molecular weight forms predominate (6, 7).

The molecule is rigid and its overall form becomes more spherical as it grows longer. It also intertwines with neighbouring coils to form a **network** at concentrations greater than 0.1%. Only around 0.1% of the volume of the molecule consists of hyaluronic acid; the remainder is water that is physically trapped inside the coil (8). A large number of hydrogen bonds are formed between the hydroxyl groups of hyaluronic acid and water, and it has been found that a gram of hyaluronic acid can hold up to 6 L of water (1).

HYALURONIC ACID IN THE BODY

A human body of 70 kg contains about 15 g of hyaluronic acid, with 50% of the total quantity being found in the skin (9). It is found mainly in the **dermis** as a primary constituent of the extracellular matrix, where its content is significantly higher than in the epidermis (10).

In addition to enhancing the skin's ability to bind water, hyaluronic acid encourages cell migration and communication between skin cells, improves tissue resistance to mechanical damage, inhibits the production of free radicals, soothes inflammation and has a regenerative effect on the skin (4, 10, 11).

Hyaluronic acid is found in large quantities in **young skin**. As we age, our bodies become less efficient at producing new hyaluronic acid. This is one of the contributing factors for skin atrophy, dryness and loss of elasticity that are typical of ageing skin (10, 12). In addition, UV radiation, pollutants and excessive stress further contribute to premature ageing through hyaluronic acid degradation (11, 13).

Hyaluronic acid has a dynamic turnover rate, with its half-life being less than a day in the skin. Hyaluronidases hydrolyse the links between N-acetyl-D-glucosamine and D-glucuronic acid residues to break down hyaluronic acid into fragments of varying lengths. Thus far, six hyaluronidases have been identified in the human body: hyaluronidases 1 to 4, PH-20 and hyaluronidase P1 (10).

Hyaluronic acid can also be **degraded non-enzymatically** by a free-radical mechanism in the presence of oxygen and reducing substances, such as thiols, ferrous or cuprous ions. Delaying this free radical degradation could mean maintaining the integrity of dermal hyaluronic acid and its moisturising properties in the skin (10).

METHODS OF MANUFACTURE

It is possible to obtain hyaluronic acid from a variety of natural sources. According to the CIR Safety Assessment of 'Hyaluronates as Used in Cosmetics', hyaluronic acid is produced through **bacterial fermentation** or **rooster comb extraction** (14). Hyaluronic acid extracted from animal tissues may contain proteins, DNA, endotoxins and chondroitin sulphate, and has the potential to provoke unfavourable immunological responses. As a result, interest in animal sources has diminished (14).

Alternatively, hyaluronic acid is obtained by *Streptococcus* bacteria through fermentation under anaerobic conditions with glucose as the carbon source. Even though the possibility of mutations or contamination by exogenous products or endobacterial toxins is low, researchers are still looking for other alternatives (4, 5, 14).

To overcome these challenges, two alternatives have been considered in scientific research. The first alternative is to manufacture hyaluronic acid using a **cell-free technology**, such as enzymes, thereby avoiding endotoxin contamination and reducing purification costs (15). These cell-free systems, however, are not yet optimised, since they provide extremely low yields, and do not represent a viable option for commercial production (16). The second alternative is to **genetically design new microorganisms** that do not produce endotoxins (17).

Hyaluronic acid possesses the same basic chemical structure regardless of its source or molecular weight. The degree of polymerisation of hyaluronic acid from different tissues, however, appears to be the primary distinction; from the bovine vitreous: 10^4–10^5 Da, from *Streptococcus* bacteria: 10^5–10^6 Da, from rooster comb: 10^6–10^7 Da (5).

COSMETIC USE

Hyaluronic acid functions as an **antistatic, humectant, moisturising, skin-conditioning and viscosity-increasing agent** in cosmetic formulations (8, 18). It is often included in products marketed as 'anti-ageing'. When solutions of high molecular weight-hyaluronic acid are applied to the surface of the skin, they result in the formation of a hydrated viscoelastic film. While at the same time it is fixing moisture to the skin's surface, the film is air-permeable and does not interfere with skin 'respiration' (8).

According to statistics from the FDA's Voluntary Cosmetic Registration Program for 2022, sodium hyaluronate has the greatest frequency of use. Sodium hyaluronate is also used in the highest concentration; it is used in face and neck products at up to 7.5% (excluding sprays). It can also be found in products applied near the eye (in eye shadows at up to 0.96%), in baby products (up to 0.005%), face powders (up to 0.099%) and other skincare preparations (in sprays at up to 0.01%) (14). Differences in the concentration of hyaluronic acid ingredients used in cosmetics on the US market in 2005 and 2021 are presented in Table 1.

Table 1: Differences in concentration of hyaluronic acid ingredients used in cosmetics on the US market in 2005 and 2021 (8)

INCI name	Range of concentrations (%)	
	2005	2021
Hyaluronic Acid	0.00005–1	0.000002–0.83
Sodium Hyaluronate	0.000001–2	0.00001–7.5
Hydrolized Hyaluronic Acid	/	0.002–0.2
Hydrolized Sodium Hyaluronate	/	0.0015–0.15
Sodium Acetylated Hyaluronate	/	0.002–0.1

MOLECULAR WEIGHT-DEPENDENT CHARACTERISTICS: SKIN PENETRATION AND COSMETIC EFFECTS

The molecular weight of hyaluronic acid plays a significant role in determining whether or not it will penetrate into epidermal skin layers. The molecule is classified depending on the chain length as:

- **small-size** hyaluronic acid fragments (HAFs; less than 50 kDa),
- **intermediate-size** hyaluronic acid fragments (HAFi; between 50 and 400 kDa), and
- **large-size** hyaluronic acid fragments (HAFl; more than 400 kDa) (19).

Literature also describes the fragments as very low-, low- or high molecular-weight hyaluronic acid. Generally, the range coincides with the aforementioned small-, intermediate- and large-size fragments, although different definitions are found by different authors. It is recommended to **check the size intervals** in the literature source in order to compare the results of scientific studies or when selecting the desired ingredient from a manufacturer or supplier.

In experimental studies, it was observed that dermally applied small-size and intermediate-size hyaluronic acid fragments **penetrate** the epidermis, but only intermediate-size fragments induced epidermal and dermal cellular proliferation *in vivo*. A vehicle-controlled study by Kaya *et al.* reported that intermediate-size hyaluronic acid fragments increased human skin thickness and clinically improved skin atrophy in patients with age- or corticosteroid-related skin atrophy. In contrast, large-size hyaluronic acid fragments and small-size hyaluronic acid fragments at the same concentration showed no impact on skin thickness (19).

In another study, 76 female subjects with periocular wrinkles received a 0.1% cream formulation containing hyaluronic acid of various molecular weights twice daily for 60 days. When compared to the placebo, all hyaluronic acid-based creams exhibited a significant increase in skin moisturising and overall elasticity values. However, a substantial decrease in wrinkle depth was only observed in women treated with a formulation containing low molecular-weight hyaluronic acid (50 and 130 kDa), which might be attributed to low molecular-weight hyaluronic acid's superior penetration ability (20).

Conventional hyaluronic acid molecules have a diameter of 3,000 nm. In terms of skin penetration, this presents a challenge for the use of hyaluronic acid in dermally administered anti-ageing products because the size prevents traditionally manufactured hyaluronic acid from penetrating the deeper layers of the dermis. Scientists at Forlle'd Laboratories in Japan were able to reduce the size of hyaluronic acid molecules to the nano range of 5 nm without affecting their subunit structure, allowing the hyaluronic acid to penetrate deeply into the dermis (21). The

anti-wrinkle effectiveness of a novel nano-hyaluronic acid in the product range of a cream, serum and lotion was then evaluated in 33 women with an average age of 45 years over the course of eight weeks. Results showed that the novel nano-hyaluronic acid exhibited a considerable reduction in the depth of wrinkles (up to 40%), and significant improvement in skin hydration (up to 96%), skin firmness and elasticity (up to 55%) (21).

When hyaluronic acid was applied dermally, the smaller the molecular weight, the greater skin penetration and skin hydration were observed in a controlled setting (20).

DETERMINING THE EXTENT OF SKIN PENETRATION

Several studies were performed with the specific aim of **determining the depth of skin penetration**. Hyaluronic acid (1350–4500 Da) was administered dermally twice daily for five days to 11 Sprague-Dawley rats. Hyaluronic acid penetrated 136 µm beneath the epidermis. In another study, radiolabelled hyaluronic acid of the same molecular weight was applied to one Sprague-Dawley rat (singular dose). After four hours, radiolabelled hyaluronic acid penetrated to a depth of 800 µm (14).

Radiolabelled hyaluronic acid with a molecular weight of 400 kDa was incorporated into a gel. Penetration was tested in one male subject (forearm, two total applications 12 hours apart; skin removed by biopsy seven hours after the last treatment). Autoradiography was utilised to detect the radiolabelled hyaluronic acid. The test substance penetrated all skin layers (22).

A skin penetration assay was carried out on full-thickness human dermatomed skin samples exposed to a 1% aqueous solution of hyaluronic acid with three different molecular weights (20–50 kDa, 100–300 kDa and 1000–1400 kDa). Water was used as a negative control and glycerol as a positive control. A total of 300 µL of the solution was deposited on the skin for eight hours at 32 °C for permeation tests. Hyaluronic acid solutions, ranging from the lowest to the highest molecular weight, were shown to be present in the epidermis at 100 µm (full epidermal depth), 50 µm and 25 µm, respectively. The majority of hyaluronic acid was observed in the stratum corneum, 25 µm from the skin surface, regardless of the molecular weight (23).

The scientific research described above is both highly desirable and informative. When interpreting the findings, however, it is important to keep in mind the **differences** between animal and human skin *in vitro* and *in vivo*, as well as a high degree of **variability** among the findings.

DERMAL IRRITATION AND SENSITISATION

Nine patients underwent a skin prick test. Each subject's forearm was pricked with sodium hyaluronate (10 mg/mL), and then examined after 15 minutes, two, six and 24 hours with no recorded skin reactions (14).

The use of dissolving microarray patches with 30% hyaluronic acid in distilled water was put through a series of safety tests on 30 people (patches were applied under the eyes three times per week for a total of four weeks). During the course of the trial, no participants experienced any adverse effects on their skin or eyes (24).

CLINICAL STUDIES

References (25) and (26) present a **comprehensive review** regarding the use of hyaluronic acid for care and treatment purposes.

Briefly, numerous preclinical and clinical studies have been conducted to investigate the potential of hyaluronic acid-based formulations (creams, ointments, gels, injections, etc.) for the treatment of a wide variety of inflammatory skin conditions. These include, for example, dry skin, skin hyperplasia, actinic keratosis, photokeratitis, acute and chronic wounds, rosacea, nasolabial folds, skin cancer, skin erythema, superficial keratitis, solar keratosis and age-related epidermal dysfunction. Hyaluronic acid-based dressings, films and grafts have also shown great potential for the treatment of various other inflammatory skin diseases (25).

Dermatological, aesthetic and procedure-focused clinical investigations using hyaluronic acid-containing products for anti-ageing benefits have also been published. In addition, a growing number of studies examine the relationship between hyaluronic acid and other face rejuvenation techniques (26).

IN FOCUS: MOLECULAR WEIGHT AND INFLAMMATORY RESPONSE

While the cosmetics industry continues to broaden the spectrum of available hyaluronic acid raw materials as cosmetically active ingredients, scientific research has opened discussions about their **possible involvement in inflammation**. It has been shown *in vitro* that high molecular-weight hyaluronic acid preserves homeostasis, inhibits inflammatory response and induces an anti-inflammatory response in a concentration-dependent manner in different cell types. On the other hand, low molecular-weight hyaluronic acid acts as an endogenous danger signal, activating inflammation (27, 28–32). The exact impact on skin cells, however, remains unknown due to a lack of research.

In vitro studies have shown that in the event of skin tissue injury, high molecular-weight hyaluronic acid is broken down into low molecular-weight fragments that **promote** inflammation. Subsequently, this causes the release of reactive oxygen species and activates enzymes (matrix metalloproteinases) that destruct the skin's extracellular matrix. Immune response through immune cells, such as CD44+ leukocytes and macrophages, is also recruited (27, 32).

Although general skin penetration characteristics of low molecular-weight hyaluronic acid have been thoroughly studied and defined, the detailed intra- and extracellular impact of low molecular-weight hyaluronic acid is not fully understood. The changes in protein profile in normal human fibroblasts induced *in vitro* by different concentrations of 20–50 kDa low molecular-weight hyaluronic acid (0.125%, 0.25% and 0.5%) have been described and quantified (33). After a 24-hour treatment, low molecular-weight hyaluronic acid increased cell proliferation, growth, extracellular matrix remodelling and proteoglycan synthesis. At the maximum concentration (0.5%), lymphocytes, interleukins (IL-12, IL-1, IL-2, IL-4, etc.) and tumour necrosis factor (TNF) stimulated inflammatory and immunological response. Global cell health was still maintained. A 0.5% low molecular-weight hyaluronic acid also increased signalling pathways involved in angiogenesis by stimulating endothelial cell proliferation, survival, migration and endothelial permeability (33). Low molecular-weight hyaluronic acid improved nucleus and mitochondrial activity, and the rearrangement of the extracellular matrix in normal human dermal fibroblasts. At the highest concentration, the cell's wellness was confirmed, although mild inflammatory and immunological activities were induced (33).

Process of wound healing – inflammation is needed

Recent research has shown the significance of hyaluronic acid chains of varying lengths in wound healing, particularly in light of the simultaneous presence *in vivo* of both high molecular-weight and low molecular-weight hyaluronic acid at an injury site (34). It has been shown that low molecular-weight hyaluronic acid with a molecular weight range of 35–280 kDa plays an important role in the propagation of an inflammatory response, through the activation of macrophages and the production of chemokines *in vitro*. Other studies have shown similar results, demonstrating that hyaluronic acid with low and intermediate molecular weight (20–450 kDa) modulated gene expression in endothelial and epithelial cells, as well as in macrophages and eosinophils (34).

An **inflammatory response** is the first signal in the activation of **wound repair** mechanisms. When it is overexpressed, however, it may promote undesirable effects (34). In the event of tissue damage, fibroblasts in the wound environment enhance the production of hyaluronic acid, while hyaluronidases produced by inflammatory cells and bacterial invaders simultaneously break down hyaluronic acid into low molecular-weight fragments (35).

Since it has been shown that these hyaluronic acid fragments trigger inflammatory cells, such as macrophages and dendritic cells to release pro-inflammatory mediators and enzymes that degrade the extracellular matrix, they have been considered a possible factor in the process of tissue damage (36).

In a study by D'Agostino *et al.* (34), stabilities to the hyaluronidase degradation of high molecular-weight hyaluronic acid, low molecular-weight hyaluronic acid and a complex of high molecular-weight and low molecular-weight hyaluronic acid, respectively, were tested for their capacity to accelerate wound healing in human keratinocytes *in vitro* and for their effect on the cellular biomarker expression. The hyaluronic acid complex showed better resistance to hyaluronidase. When compared to either high molecular-weight hyaluronic acid or the hyaluronic acid complex, low molecular-weight hyaluronic acid (90 kDa) was more successful in improving the wound reparative process. It has been proposed that smaller molecules (ranging from 5 to 20 kDa) are promoters of the early inflammatory response, which is essential for the early stage of wound healing. Finally, the authors concluded that low molecular-weight hyaluronic acid proved not to be toxic or inflammatory, and it accelerated wound repair at an earlier stage, while high molecular-weight hyaluronic acid had no short-term effect.

Further research on skin models

Research by M. Farwick *et al.* (36) studied very low molecular-weight hyaluronic acid's effect on keratinocytes. They aimed to identify the low molecular-weight hyaluronic acid with excellent anti-ageing and moisturising effects, efficient skin penetration and no inflammation-related negative effects. The reconstituted human epidermis was incubated for 48 hours with aqueous solutions containing 0.5% 20 kDa, 50 kDa, 130 kDa and 320 kDa hyaluronic acid, respectively. TNF-α expression was used as an indicator of pro-inflammatory response. Hyaluronic acid with a molecular weight greater than 50 kDa had no significant effects, while the use of very low molecular-weight hyaluronic acid (≤ 20 kDa) may not be safe due to the initiation of an inflammatory response in keratinocytes.

Another set of experiments characterised the effects of hyaluronic acid on human epidermis skin models; 0.5% aqueous solutions with 50 kDa and 800 kDa hyaluronic acids, respectively, were applied dermally to the reconstructed human epidermis for 48 hours. As a result, 800 kDa hyaluronic acid induced the expression of 40 genes and 50 kDa hyaluronic acid induced the expression of about 120 genes (genes involved in keratinocyte regulation and genes that are important for the development of tight junction complexes). There was no sign of an inflammatory reaction, leading to the conclusion that hyaluronic acid larger than 50 kDa is safe to use (36).

EVIDENCE-BASED FORMULATING

Physical form

Hyaluronic acid comes in the form of a highly purified, freeze-dried powder or an aqueous solution, and as potassium or sodium salt (14). Sodium hyaluronate comes in the form of a white, fibre-like or creamy white powder with a very slight odour (8). Manufacturers and suppliers typically specify a range of molecular weights.

Viscosity

In water, hyaluronic acid dissolves slowly but completely, producing solutions that are viscous, transparent to slightly opalescent and colourless. These

solutions must be preserved for cosmetic use. Depending on the preparation process, viscosity might vary and drop significantly in the presence of electrolytes (8).

Sodium hyaluronate in water forms a clear to faintly opalescent colourless and viscous solution. It dissolves slowly but completely. It is almost insoluble in organic solvents (up to 50% alcohol-water mixtures depending on the alcohol type and the molecular weight of the sodium hyaluronate). As the substance is a good source of nutrients for bacteria, aqueous solutions of sodium hyaluronate must be preserved, too (8). Molecular weight affects dissolving speed, where a lower molecular weight typically means faster dissolving.

pH

The pH of a 0.2% water solution of sodium hyaluronate can range from 5.5 to 7.5. The structure is pH- and ionic strength-dependent. At a physiological pH of 7, carboxyl groups of the hyaluronic acid molecule fully dissociate. Due to repulsions between charges, the molecule expands at a lower ionic strength. This increases viscosity (8).

Skin hydration and TEWL in a gel or emulsion vehicle

As a skin hydration vehicle, emulsions of hyaluronic acid were shown to have better skin hydration results than gels (concentration 0.1%, molecular weight 58.2 kDa). With respect to transepidermal water loss (TEWL) in comparison with the same concentration of hyaluronic acid in emulsions, 0.1% of the 58.2 kDa and 0.05% of the 58.2 kDa hyaluronic acid in gels had higher TEWL values measured after one hour of application (37).

Types of cosmetics

Sodium hyaluronate is found in products for skin care, e.g. products for an ordinary facial routine, pre- and after-shave products and sun care products, repair cosmetics and lipsticks, in skin cleansers, hair care products, etc. (38).

Recommended concentrations

In most clinically studied cases, the concentration range was 0.1 to 0.2 % (w/w) (25, 26). It thus makes sense to use the same concentrations when formulating active cosmetics in order to achieve the desired cosmetic effects.

Effect on the penetration of other chemicals

Because a hydrated epidermis can be more permeable, hyaluronic acid could increase the penetration of other substances through the human stratum corneum (8). The Cosmetic Ingredient Review Expert Panel (8) concluded that the presence of hydrating substances might increase the amount of other compounds that are absorbed by the skin. According to the Panel, care should be taken when developing cosmetic products with ingredients that have a lack of dermal absorption data or where dermal absorption is a concern.

Compatibility and processing

Sodium hyaluronate is relatively stable. Changes in molecular weight can occur at extreme pH levels or during heating (a higher molecular weight means lower stability). Experimental data show that temperatures of up to 50 °C are considered acceptable, but a significant decrease in stability occurs at 60 °C and above (39).

Sodium hyaluronate is sensitive to free radicals, and forms insoluble salts with cationic substances (e.g. cationic detergents) and high molecular-weight positively charged substances (e.g. chitosan and quaternised polymers. Please note that many of these ingredients may not be allowed in natural cosmetic certifications) (40).

A 2% hyaluronic acid solution typically retains water so tightly that it forms a firm gel. Larger hyaluronic acid molecules aggregate and entangle more than their smaller fragments (41, 42).

Moisturising creams (oil-in-water emulsions) may be more suitable than water-in-oil emulsions as vehicles in terms of hyaluronic acid skin penetration, as concluded in a long-term stability testing of four emulsions. Both the migration of particles and flocculation were observed in water-in-oil formulations. The higher the water content, the more stable the formulation can be due to the hygroscopic properties of hyaluronic acid (43).

CONCLUSION

Hyaluronic acid is a widely used cosmetic ingredient with proven effects on skin hydration. It is non-irritating and non-toxic when applied to the skin. Depending on the molecular weight it serves different purposes in cosmetic products.

Low molecular-weight hyaluronic acid triggers inflammation, but inflammatory and immunological activity is well tolerated in cell cultures. Based on the presented findings, it is considered a **relatively safe** ingredient. Hyaluronic acid with a molecular weight above 50 kDa does not cause inflammation process in the skin and is therefore **safe** to use.

Although low molecular-weight hyaluronic acid shows promise in early-stage wound healing, **more research should be done** on its use in cosmetic products and its effects on the skin due to its superior penetration abilities.

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Supercritical CO_2 extraction for plant extracts: Basic facts and challenges for cosmetic formulating

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ABSTRACT

The alternative extraction of compounds from natural sources is the most studied use of supercritical fluids. Supercritical CO_2 extraction has important advantages over extraction methods that use traditional organic solvents, particularly in terms of environmental impacts. The process is also extremely flexible due to the possibility of continuously modulating solvent strength or the selectivity of the supercritical CO_2. Last but not least, the separation of solvent and extract is simple. For all these reasons, CO_2 extracts have gained popularity in cosmetics as environmentally friendly, safe and potent cosmetically active ingredients of natural origin.

Keywords: essential oil, green extraction, plant extract, supercritical CO_2, vegetable oil

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INTRODUCTION

Throughout the history of the cosmetics industry, **ingredients of natural origin** have been the cornerstone of both natural and conventional cosmetic products. Their benefits are unquestionable, particularly in terms of dermal and environmental impacts. As raw materials, we can use whole plants or plant parts (e.g. herbal substances such as ground coffee beans for peeling) and isolated compounds (e.g. vegetable oils, tocopherol or bakuchiol), but the most frequently used are **plant extracts**.

Plants represent the oldest source of cosmetically active ingredients. Therefore, many traditional **extraction procedures** are in use with the aim of concentrating cosmetically active ingredients that are mostly present in plants in small concentrations (1). However, scientists are still intensively engaged in the development of more efficient and selective extraction methods.

One of the new, so-called green extraction technologies is supercritical fluid extraction, where carbon dioxide (CO_2) is typically used as a solvent. In this article, we focus on the advantages and disadvantages of **supercritical CO_2 extraction**, and the three important types of extracts that are obtained using this method and that are of great value as cosmetic ingredients.

SUPERCRITICAL CO_2 – WHAT DOES IT MEAN?

Supercritical fluid extraction is a special concept that uses solvents in a supercritical state. A supercritical state means that the conditions of the extraction environment are above the solvent's **critical point**, i.e. above the critical temperature and critical pressure (Figure 1). The critical temperature is the highest temperature at which a gas is converted into a liquid through an increase in pressure, while the critical pressure is the highest pressure at which a liquid is converted into a gas through an increase in temperature (2–4).

During this transition, **unique changes** in the properties of matter occur. The supercritical state corresponds to the region where the physico-chemical properties, such as density, viscosity, diffusion coefficient and thermal conductivity, are between those of a liquid and a gas. In a critical state, the liquid and gas phases are equal and homogeneous, as the substance exhibits liquid-like density and gas-like viscosity and diffusivity, which facilitates good mixing and mass transfer (2–4).

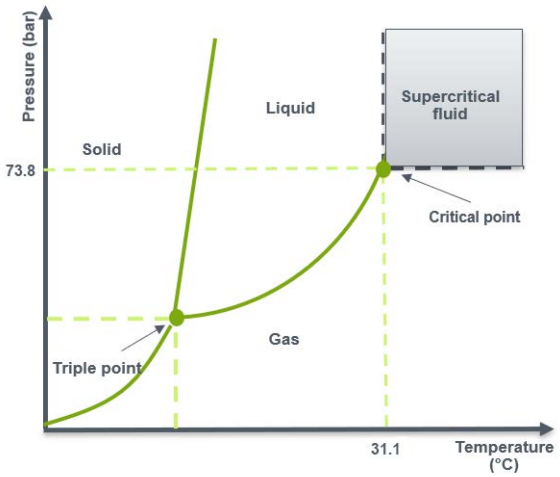

Figure 1: Phase diagram of CO_2.

Theoretically, several organic solvents and gases are suitable for use in a supercritical state. However, **CO_2 is most frequently used** due to its non-carcinogenicity, non-toxicity, non-mutagenicity, non-flammability, thermodynamic stability, affordability and easily achievable supercritical conditions (3).

At the same time, **CO_2 is generally recognised as a safe solvent** by the EFSA (referring to extraction solvents that are used in the processing of raw materials, the production of foodstuffs, and the ingredients or impurities thereof according to Directive 2009/32/EC) and the FDA (referring to the generally recognised as safe or GRAS status) (4–6).

SUPERCRITICAL CO_2 – WHY IS IT BETTER?

Technologically speaking, **traditional extraction processes** are mostly simple and well-established methods. However, they are associated with some important disadvantages: large amounts of waste solvents, which are often toxic and flammable, and the use of heat, which can lead to the breakdown of temperature-sensitive compounds (5, 7).

On the other hand, CO_2 extracts are obtained by extraction using supercritical CO_2, and CO_2 is a gas that achieves the objectives of traditional extraction processes. It acquires the properties of a liquid at a pressure of 73.8 bar and a temperature of 31.1 °C. It thus acts as a solvent and, due to its non-polar nature, extracts mainly **lipophilic (non-polar) molecules** from a plant. At the end of the extraction process, when the pressure is reduced, solvent saturated with the extract goes into a separate collector where CO_2 returns to a gas state and completely disappears from the extract, and only pure extract remains. Extraction technology was described in detail by Dr. Marko Likon in Volume 1 of the CosmEthically ACTIVE Journal (8).

We use supercritical CO_2 to extract biologically active compounds from **all plant parts**: from seeds, fruits, flowers, leaves, stems and bark, to rhizomes and roots. This method facilitates the extraction of **various bioactive compounds**, including triglycerides, fatty acids, fatty alcohols, volatile terpenes and terpenoids (better known as constituents of essential oils), phytosterols, tocopherols and tocotrienols, carotenoids and phenols. Finally, CO_2 extraction is currently most relevant for the extraction of **essential and vegetable oils** (13, 14).

KNOW YOUR EXTRACTS

The path from a plant to a plant extract begins with an **herbal substance** (also known as an herbal drug). Plant extracts are made for various reasons, such as the removal of toxic and inactive compounds, increasing the stability of bioactive compounds, and reducing volume and improving appearance, smell and taste. Plant extracts have advantages over pure isolated compounds in terms of **synergistic action** and the reduction or prevention of unwanted effects of individual compounds. On the other hand, the complexity of their composition results in difficult evaluation (11).

In general, two key factors are important for obtaining a **high-quality extract**: a high-quality herbal substance, and an optimised and well-defined manufacturing process. Furthermore, at the level of extraction, the composition of an extract is significantly influenced by temperature, extraction solvent and the ratio between an herbal substance and a solvent. In addition, an important piece of information for cosmetic formulators is the ratio between an herbal substance and the final extract obtained, also known as the **DER ratio** (11).

To simplify the explanation, if we have marigold flower extract with a DER of 10:1, this means that a manufacturer made 1 kg of the extract from 10 kg of marigold flowers. DER therefore defines the content of cosmetically active ingredients in an extract and, thus, affects the appropriate concentration of the extract we are going to include in a formulation to achieve an effective **cosmetic activity**.

There are also other criteria that make an extract a high-quality extract. In the manufacturing process, the absence of impurities must be ensured (e.g. pesticides, heavy metals, microorganisms and residues of organic solvents). Following the extraction process, the **content of cosmetically active ingredients** or, in a wider sense, the content of bioactive compounds in the final extract must be determined using an appropriate analytical method (11). This content is usually found in raw material **specification documents** such as a certificate of analysis (CoA) and technical datasheet (TDS).

When producing plant extracts of **pharmaceutical quality**, manufacturers must consider and accurately define all the factors emphasised above. In the areas of cosmetics, food supplements or food, however, the requirements are not so strict and well-defined. We are therefore faced with the issue of **how to recognise an extract as high-quality** (11). Understanding of basic principles about extraction methods and the extraction-related factors mentioned above are therefore of great importance.

SIMILARITY TO ESSENTIAL OIL, VEGETABLE OIL OR MACERATE?

The extraction of compounds from natural sources is the most commonly studied application of supercritical CO_2 (4, 12). From an historical point of view, the first uses of supercritical CO_2 extraction on an industrial scale were for the decaffeination of tea and coffee, and later for the extraction of hops constituents (4, 5).

In general, there is a great deal of confusion when it comes to **distinguishing CO_2 extracts from traditional extracts** and using them as raw materials. The term 'CO_2 extract' can refer to different types of extracts, e.g. 1) aromatic CO_2 extracts that represent a chemical alternative to essential oils, 2) oil CO_2 extracts that represent a chemical alternative to fatty oils and 3) other CO_2 extracts that are typically rich in specific compounds such as carotenoids or tocopherols. The latter are frequently referred to as 'super potent' macerates. These three types of CO_2 extracts are discussed below.

Aromatic CO_2 extracts – alternative for essential oils

Essential oils represent only a small part of the composition of plants: up to a few percent for plant material with a high content of volatile compounds, such as citrus peels, or below 0.1% for rose petals (13). Essential oils are known for their **complex composition**, which includes several to several hundred components. Chemically, they are classified as terpene and sesquiterpene hydrocarbons and their oxygenated derivatives (e.g. aldehydes, alcohols, ketones ethers and esters). They determine the smell and aroma of essential oils. In addition to their aromatic properties, essential oils also have antioxidative, antimicrobial, anticancer, anti-inflammatory and anti-viral properties. The largest amount of pharmacological data comes from *in vitro* and animal *in vivo* studies. We can, however, also find clinical studies conducted on humans (14).

It should be emphasised that, by definition, essential oils are **volatile compounds** obtained through distillation and pressing. **Pressing** is only suitable for the production of essential oils from citrus plant species. Technically speaking, **distillation** is a simple process, but has many disadvantages: during the process, thermal decomposition, hydrolysis and dissolution of water-soluble compounds can occur, which change the smell of a naturally present plant scent (11). All of these are the reasons why CO_2 extraction is used today with great success for the extraction of essential oils.

The special value of aromatic CO_2 extracts is that they closely capture the **real scent of a plant**. They are much more similar to the plant scent itself than essential oils, as chemical reactions that occur during the distillation process do not occur here. It is worth mentioning that although essential oils contain chemically the same or similar profile of volatile compounds as aromatic CO_2 extracts, the term 'essential oil' for extracts obtained by extraction with supercritical CO_2 is not appropriate due to the above-mentioned facts (11).

Another advantage of CO_2 extraction over distillation is that CO_2 extracts are richer in composition, as they also contain **non-volatile compounds**. This is also the reason that these extracts can appear different in terms of consistency, as they can be viscous or even semi-solid. Volatile compounds are extracted together with, for example, waxes that are present in the cuticle on the surface of plant material, and the extract thus loses its liquid form. Last but not least, many non-volatile compounds express beneficial skin effects (1, 12 ,15).

Oil CO_2 extracts – Alternative to vegetable oils

The supercritical CO_2 extraction method is also useful for the isolation or fractionation of plant lipids and their processing into industrial products (6). **Unrefined vegetable oils** isolated using supercritical CO_2 are considered to be of higher quality. The first reason lies in the broader spectrum of compounds extracted. For example, a higher content of several antioxidants can be extracted through optimised extraction, such as polyphenols and tocopherols, which work synergistically. Compared to the extraction of vegetable oils using organic solvents or cold pressing, the extraction process takes place at the lowest temperature and in an environment with limited access to oxygen, which additionally helps to maintain their proper oxidative condition (18). Such an extraction method is particularly important for the isolation of unstable vegetable oils such as linseed oil (19).

Furthermore, extraction conditions can be optimised to increase oil yield, tocopherol (1) or squalene content (16).

Other CO_2 extracts

This section focuses on the last type of CO_2 extracts. They are most frequently characterised by a high content of specific compounds typical for a specific plant, e.g. antioxidants. In terms of activity, extracts obtained using supercritical CO_2 have been shown to have similar or better antioxidative properties than traditional extraction procedures, despite lower yields of CO_2 extraction. The reason lies in the broader spectrum of extracted antioxidative compounds that usually act synergistically (1).

Explained below are a few common yet very current examples. A popular CO_2 extract that is easily available as a cosmetic ingredient is a **marigold** (*Calendula officinalis*) flower CO_2 extract (17). Compared to a traditionally made extract, i.e. marigold macerate made using a vegetable oil, such as olive or sunflower oil, a CO_2 marigold extract contains a significantly higher concentration of extracted compounds, including carotenoids. To simplify the explanation, using a CO_2 extraction is similar to extracting compounds from marigold flowers, where the final extract is without an oil base, i.e. the final extract is pure marigold flower compounds.

Another popular plant for CO_2 extraction is **hemp** (*Cannabis sativa*). All three types of CO_2 extracts can be obtained from hemp. An extract with cannabinoids suits the category of other CO_2 extracts, and is rich in cannabidiol (CBD) and other cannabinoids. The oil CO_2 extract goes to the second category, as it resembles a vegetable oil in composition, and the rarely found aromatic CO_2 extract goes to the first category, as it contains volatile compounds from hemp and resembles the composition of an essential oil (18).

There are many other CO_2 extracts available: hops (*Humulus lupulus*), ginger (*Zingiber officinale*), coffee (*Coffea arabica*), arnica (*Arnica montana*), guggul (*Commiphora wightii*), chilli (*Capsicum annuum*), schisandra (*Schisandra chinensis*), etc. (19, 20).

CONCLUSIONS FOR EVIDENCE-BASED FORMULATING

CO_2 extracts have become extremely popular during the last decade in the food, fragrance and flavour, pharmaceutical, and food supplement sectors, and in the cosmetics industry. Indeed, extraction using supercritical CO_2 is an efficient green method with an excellent adaptation of process parameters for the extraction of desired (non-polar) compounds from plant material.

The challenge of how to identify different CO_2 extracts and, taking it a step further, how to identify high-quality CO_2 extracts is sometimes impossible to overcome, as manufacturers and suppliers often do not provide all the necessary information in the raw

material specifications of these products (this is an issue when the products are not pharmaceutical grade plant extracts).

Given below is **key information** that defines an extract and is crucial in the evaluation of the quality thereof (11):

- the identification of a plant and herbal drug – botanical name and plant part,
- the type of extract,
- the content of an extract in a raw material (when solvents or other excipients are present),
- the content of cosmetically active compounds or analytical markers in an extract, and
- the extraction method and procedure, extraction solvent, the ratio between extraction solvent and extract, and the DER ratio.

Certificates of analysis and production specifications represent a great added value when evaluating CO_2 extracts. On the other hand, aspects of **biological activity**, including **cosmetic effects**, are determined not only by the complexity and identification of an extract's composition, but also by the availability of data at the level of research on plant metabolites and, as the most reliable criterion, by the availability of data on clinically proven effectiveness. In terms of CO_2 extracts, **clinical effectiveness** for cosmetic use is expanding every day, but is still not comparable with the knowledge we currently have regarding traditional extracts.

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Unsaponifiable compounds – The overlooked cosmetic ingredients

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ABSTRACT

Unsaponifiable compounds are components found in unrefined vegetable butters and oils. Their composition is complex, and includes terpenic and aliphatic compounds, waxes, tocopherols and tocotrienols, phospholipids and phenolic compounds. Unsaponifiable compounds have been proven to act as antioxidative, anti-inflammatory, antitumour, immunomodulatory and antimicrobial agents. They express wound healing, anti-acne and anti-dermatitis activities, and hydrating, photoprotective and anti-wrinkle activities. Although no systematic studies regarding the dermal use of unsaponifiable compounds have been performed yet, available research proves that it makes sense to recommend the use of unrefined vegetable butters and oils, as well as unsaponifiable compounds alone, in cosmetics.

Keywords: skin, unsaponifiable compounds, unsaponifiables, vegetable butters and oils

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INTRODUCTION

Vegetable butters and oils are widely used in the cosmetics industry as active ingredients, ingredients of the lipid phase and other excipients. Chemically, they are a mixture of triglycerides (esters of glycerol and fatty acids) and **unsaponifiable compounds** (1). The dermal effects of vegetable butters and oils include hydrating, emollient, antimicrobial, antioxidative and anti-inflammatory activities (2). The triglyceride fraction has been researched intensely, while significantly less scientific work has been performed on unsaponifiable compounds.

By **definition**, unsaponifiable compounds are components that are not saponified when treated with alkali (NaOH, KOH), are soluble in lipids, are not volatile at 103 °C and can be extracted using organic solvents (3–5). The content of unsaponifiable compounds in vegetable butters and oils usually varies between 0.3 to 2% (6). Their **composition** is complex, and includes terpenic and aliphatic compounds, waxes, tocopherols and tocotrienols, phospholipids, phenolic compounds and traces of some specific chemical families (Figure 1) (1, 5).

The content and composition of unsaponifiable compounds is specific to a vegetable butter or oil, but varies depending on the origin and the quality of plant material, the extraction method, exposure to refining and the storage conditions of a butter or oil (exposure to air, temperature and microbial contamination) (7, 8).

DERMAL EFFECTS

There have been no comprehensive systematic studies performed in the area of unsaponifiable compounds to date, and scientific evidence about their effects after dermal application for therapeutic and cosmetic use is incomplete. Here, the **dermal effects of unsaponifiable compounds** are summarised based on our review article published in 2021 (9).

Phytol was found to have cytotoxic, autophagy- and apoptosis-inducing, anti-inflammatory, immune-modulating, antioxidative, antimicrobial and antinociceptive effects important for dermal activity. It also showed potential in addressing skin hyperpigmentation, as it decreased melanin production.

Squalene on the human skin functions primarily as an antioxidant and contributes to proper skin hydration. It acts as an anti-inflammatory agent, inhibits tumour-promoting action, contributes to faster wound healing, and was linked to beneficial effects on skin with acne and seborrheic dermatitis.

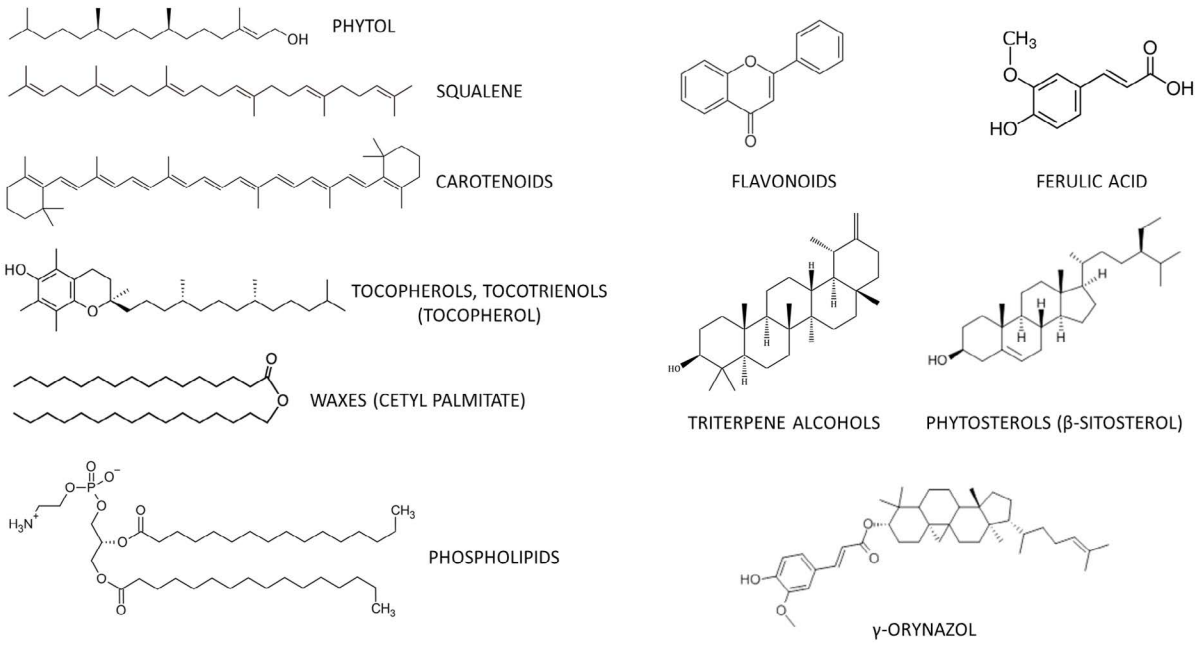

Figure 1: Structural formulas of unsaponifiable compounds described in the 'Dermal effects' section.

Triterpene alcohols exhibit anti-inflammatory, antitumour, chemopreventive and antimycobacterial properties.

Phytosterols were found to possess antioxidative, anti-inflammatory and antitumour properties, to stimulate the synthesis of hyaluronic acid and increase epidermal thickness. They were also proposed for use in hair care due to their hair-softening and hair-conditioning properties. β-sitosterol increases the effect of vitamin D and promotes wound healing due to its angiogenic activity.

The beneficial dermal effects of **carotenoids** are due to their antioxidative effects, and photoprotective, anti-inflammatory, antitumour and immunostimulative effects.

The photoprotective activity of **vitamin E** includes decreased erythema, decreased tumour incidence and decreased lipid peroxidation, and improvements in wrinkled skin.

Flavonoids were shown to have significant antioxidative activity, and they also act as antibacterial, antiviral and anti-inflammatory agents.

Ferulic acid was shown to have antioxidative activity in keratinocytes, fibroblasts and animals, as well as wound healing, photoprotective and antimelanogenesis activities, while it also ameliorated symptoms of atopic dermatitis on mice skin.

Most known dermally applied **wax** is wax of jojoba seeds, which has been shown to accelerate the wound closure, stimulate collagen synthesis, and express hydration, antioxidative, anti-inflammatory and antibacterial effects.

γ-Oryzanol (a mixture of esters of ferulic acid and triterpene alcohols or sterols extracted from rice bran oil) has an antioxidative activity. A 1% γ-oryzanol ointment was observed to cause an increase in sebum production.

Phospholipids are the dominant lipids in cell membranes, and lysophosphatidic acid was reported to be beneficial in wound healing, pruritic skin disease, skin tumours, scleroderma and skin inflammation reaction.

To recap: isolated unsaponifiable compounds possess **antioxidative**, **anti-inflammatory**, **antitumour**, **immunomodulatory** and **antimicrobial activities**, **wound healing**, **anti-acne** and **anti-dermatitis activities**, as well as **regenerative**, **hydrating**, **photoprotective** and **anti-wrinkle activities**.

CLINICAL STUDIES

Knowledge about the specific mechanisms of action of isolated unsaponifiable compounds helps us understand their general benefits when used dermally, either alone or as ingredients in cosmetic formulations. It should be emphasised, however, that the results of *in vitro* and *in vivo* animal research cannot be directly extrapolated as real effects after application on the human skin (9). For this reason, **clinical studies** are needed and are of great importance for the better understanding of the dermal effects of unsaponifiable compounds.

Two clinical studies researching dermal effects of unsaponifiable compounds are summarised below.

Unsaponifiable compounds of canola (*Brassica* sp.) oil and shea (*Vitellaria paradoxa* syn. *Butyrospermum parkii*) butter

The effects of dermally applied substances (canola oil, unsaponifiable compounds of canola oil, sunflower oil, borage oil, fish oil, petrolatum, water and 1% hydrocortisone cream) were investigated in a study by Lodén et al. (10). Seven men and 14 women (aged 22 to 75 years) were included in the study where 50 µL of each investigated substance were placed in aluminium chambers and attached to the skin on the volar surface of the forearm. First, the skin was exposed to a 14% aqueous solution of sodium lauryl sulphate (SLS) for seven hours, and then the tested substances were applied for 17 hours. No pre-treatment with SLS was performed on the control arm. Skin areas were examined after 24 hours. Shea butter, sunflower oil, petrolatum and water induced very weak, barely perceptible erythema on the control skin. On the SLS-treated skin, canola oil, canola oil unsaponifiables and hydrocortisone **reduced transepidermal water loss** (TEWL) significantly, while canola oil unsaponifiables and hydrocortisone **reduced blood flow** significantly compared with exposure to water. The effects of other substances were insignificant.

Unsaponifiable compounds of hazelnut (*Corylus avellana*) oil

A study by Masson et al. (11) was conducted with virgin hazelnut oil (containing 286 ppm of phospholipids), refined hazelnut oil (phospholipids in traces) and refined hazelnut oil enriched with previously extracted phospholipids (224 ppm of phospholipids). Each oil was incorporated at a concentration of 10% into a test (an emulsion) and the control emulsion contained no hazelnut oil. Four groups of subjects (56 women aged 30 to 45 years) applied the test emulsions and the control emulsion on the forearm skin twice a day for 28 days. Results showed a significant **hydrating effect** for all emulsions. The differences between virgin and enriched oil emulsions were not significant, but the effect of the emulsion with virgin oil was statistically significant relative to the emulsion with refined oil. The identified differences in hydration properties thus related to phospholipids.

EVIDENCE-BASED FORMULATING

Due to their chemical composition, unsaponifiable compounds are **soluble in lipids** and should therefore be incorporated in the lipid phase of a cosmetic product.

In contrast to a large number of vegetable butters and oils available, only a few unsaponifiable compounds have been reported to be used as cosmetic ingredients in dermal formulations (14):

- unsaponifiable compounds from shea (*Vitellaria paradoxa* syn. *Butyrospermum parkii*) butter,
- canola (*Brassica* sp.) oil,
- soybean (*Glycine max* syn. *Glycine soja*) oil,
- sunflower (*Helianthus annuus*) oil,
- olive (*Olea europaea*) oil and hydrogenated olive oil,
- avocado (*Persea americana* syn. *Persea gratissima*) oil,
- sesame (*Sesamum indicum*) oil, and
- corn (*Zea mays*) oil.

Similarly, very little data exist about **recommended or cosmetically active concentrations**. According to a safety assessment of plant-derived fatty acid oils (12):

- 0.19% of soybean oil unsaponifiable compounds were incorporated in a face and neck product;
- 2% of sunflower seed oil unsaponifiable compounds were incorporated in a night product;
- 2% of sunflower seed oil unsaponifiable compounds were incorporated in a face and neck product;
- 2% of olive oil unsaponifiable compounds were incorporated in a face and neck product; and
- 5% of olive oil unsaponifiable compounds were incorporated in a skin cleansing product.

None of these unsaponifiable compounds were found as dermal irritants or sensitisers (12).

In research regarding the biochemical effects of unsaponifiable compounds of avocado and soybean oil on hairless rat skin, 5% of unsaponifiable compounds were dissolved in almond oil. A beneficial effect on the metabolism of skin collagen was suggested, as an increase in the concentration of skin soluble proteins and soluble collagen was seen (13).

FUTURE PROSPECTS

Unsaponifiable compounds were reported to significantly improve wound-healing action, together with the action of fatty acids of triglycerides (2). It therefore makes sense to recommend the use of **unrefined vegetable butters and oils**, i.e. to avoid the refining process and preserve unsaponifiable compounds.

Although unsaponifiable compounds are considered safe for cosmetic use, the importance of detailed further investigation was emphasised, in particular, in terms of the **potential permeation enhancement** of other ingredients applied to the skin (15), as cosmetic products are typically used daily and over longer periods of time.

The dermal use of unsaponifiable compounds in the treatment of skin disorders is largely unexplored, but has also been recognised as promising according to two patents (16, 17).

CONCLUSION

Based on the reviewed *in vitro* and *in vivo* studies, unsaponifiable compounds contribute significantly to the overall dermal effects of vegetable butters and oils. Although direct evidence is limited, it is reasonable to expect that the **antioxidative effects** of unsaponifiable compounds will be expressed on the skin's surface and in the epidermis. Similar conclusions can be drawn for **antimicrobial effects**. Dermally applied unsaponifiable compounds (phytosterols) were found to beneficially affect **the function of surfactant-irritated skin**, improve the **elasticity** of animal skin and improve *in vitro* **collagen synthesis**. **Hydration** was shown to be significantly improved by unsaponifiable compounds in a human clinical study.

Unsaponifiable compounds are considered to be **safe** in the concentrations and practices of use in cosmetics. Clinical studies prove that they express beneficial cosmetic and therapeutic effects after dermal application. It thus makes sense to use unrefined vegetable butters and oils, as well as unsaponifiable compounds alone.

Research in the area of the dermal application of unsaponifiable compounds is still very rare, and we are looking forward to new in-depth studies in the future.

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MEET THE PROPULSIVE MESSENGERS OF THE CosmEthically ACTIVE COSMETICS ERA – Luxe Botanics and Mylo

Nina Kocevar Glavac

Two women, two personalities, two visions, two interesting stories and many exceptional CosmEthically ACTIVE certified products: discover the world of Ms. Jene Roestorf, the founder of Luxe Botanics, and Ms. Barbora Gazová, the founder of Mylo.

In the interviews, you will read about the path they walked. How did they manage to do the formulating work and testing to achieve the best-performing product? What are the key challenges and what are their future plans? And the main milestones? You will also read how the CosmEthically ACTIVE certification helps them to stand out.

There is one particular thought that touched me deeply and reminded me that we stand together for the same cause. And that is…

To be respectful of our most important partners, our customers. Indeed, they deserve to know that 'there is a true, meaningful reason behind creating a product that creates value'. Not the money, not the fame but a true wish for a healthy planet through sustainable consumption habits and nature-friendly cosmetics.

It is a privilege and honour that we are co-creating cosmetics with such dedicated people. You truly deserve my deepest admiration!

About LUXE Botanics

Interview with Jené Roestorf, the founder

Launch year

2017

The uniqueness of LUXE Botanics

LUXE Botanics is inspired by my South African roots, growing up surrounded by nature and its incredible properties. I'm also a biotechnologist with a 10-year career spanning lab bench research and clinical trials, so our range is a culmination of the most astounding, science-backed botanicals, powered by innovative biotech to create hyper-targeted solutions for your skin. Award-winning and results-driven, we are an eco-luxury skincare brand that is at the forefront of beauty activism, raising the bar for sustainable and ethical ingredient sourcing, while creating a meaningful impact among our harvesters in Africa and Brazil, and rural communities around the world.

Target audience

Our target audience is women 35+, well-educated and informed, nature-loving, animal-protecting and adventure-seeking. The LUXE woman is a health and wellness advocate, social activist and is curious about science, always doing her own research. Inspired by progress, she values quality and results in her skincare.

The most important milestone(s)

Spending nine months on R&D before our soft launch in 2016, researching truly unique botanicals with transformational benefits for the skin and ingredients that would enhance these properties.

Being selected for the Neiman Marcus Indie Beauty Expo edit during our first year and learning what it takes to be successful in a large luxury department store. Taking that feedback and channelling it into improvements on our packaging, marketing and sales support team.

Rebranding in 2019 to create a more elevated, luxury aesthetic to match the performance of our formulations and then growing into a global brand sold at 40 retailers in 14 countries.

Key challenge(s)

Cash flow. Whether you're a big or small business it's a constant juggling act.

Rising marketing costs. While skincare should be about formulations and their performance, the industry is mostly about marketing. Brands that have more money to capture people's attention have a higher probability of success. As a small brand, we rely on discerning customers who aren't driven by hype, but look to science and tried, tested and true ingredients that deliver results and an authentic story.

Doing the right thing and running a sustainable business that meaningfully gives back is costly. Because we don't cut corners and ensure we are developing skincare that creates value for everyone, it has meant raising prices in line with the true cost of our ingredients and paying harvesters a fair wage for their crops. It's not something we ever compromise on.

Formulation work

How do you start formulating a new product?

There are two things we consider:

Marketing: Polling customers, retailers, influencers, conducting customer interviews, competitor reviews, up-and-coming ingredients and products to inform the next product.

Science: Researching ingredients, finding communities that we can uplift through our botanicals, creating our formulations with a cosmetic chemist.

This is why we have ten formulations in development that we can deploy at various times on different markets to suit their needs.

How many versions of your best-performing product did you make in the formulation phase?
We made twelve iterations of our Kigelia Corrective Serum. It's an incredibly hard ingredient to work with as the fruit has to be cut down, dried, sent to a lab in the UK to be reconstituted with water and glycerin, and then we have to get it to disperse evenly in the formulation.

Is there something that you would have done differently if you were starting again?
I would have started with fewer products! I was so excited to create a whole line that I got ahead of myself from a business sense and should have launched with less, so I didn't have to split the marketing efforts across eight products. Although in hindsight this has worked to our advantage, as we are now being considered for spas, which is why we are expanding the line. I would have also looked for a partner earlier; those first couple of years were hard and lonely until my business partner Rachel came on board.

CosmEthically ACTIVE certification

Where do you see the main added value for your cosmetics line? Who would you recommend the certificate to?
I truly love the green beauty industry we're in, but often I find myself asking, where is the science? And it must be even harder for the customer to decipher what's going to make a difference to their skin. The CosmEthically ACTIVE certification is the only accreditation that considers efficacy of a natural product, in addition to ingredient origin and potential harm to the planet. I would recommend this to founders who are truly committed to efficacy, ethical formulations and ethical marketing who can back up their claims.

What was your experience with the certification process?
It took us three years to achieve our certification, as we started the process in the middle of our rebrand.

Because of this, it took us longer, but it was truly a collaborative experience. The Modern CosmEthics team was extremely rigorous and committed to reviewing every ingredient update and formulation. I would say that it resulted in the development of our most active formulations yet. What I loved most was working with real experts willing to share their extensive knowledge. They will be part of any new formulations we create (stay tuned!).

About the founder

Which part/characteristic of you is imprinted in the story of LUXE Botanics?

So many! My love for nature and fascination with science, growing up in South Africa seeing the injustice of apartheid, and wanting a business that would create equality, partner with communities and give back. Coming from a family of entrepreneurs, my talent and ambition not being appreciated in the pharma industry, going on a life-changing trip to Africa and realising I had to do something that would bring me back to my home, nature and science, and that could benefit others.

Who has been the most indispensable person on LUXE Botanics' journey?

My business partner, Rachel.

How do you find the proper work-life balance?

I wake up early, don't check my phone before I get to my desk, meditate and workout as often as I can, and turn my phone off at 10 pm.

How do you envision cosmetics in 10 years?

I hope brands will be more transparent with their customers. They deserve to know who is making their products and why. Is it just for the money or the fame, or is there a true, meaningful reason behind creating a product that is creating value?

A change we're already starting to see is one towards science, not away from it, and a backlash against using words such as lab-made and synthetics in a negative, scaremongering way in marketing. I see us embracing cutting-edge green technology that is and will become more widely available to us to create active, thoughtful formulations that also meaningfully care for people and our planet.

About Mylo

Interview with Barbora Gazová, the founder

Launch year
2013

The uniqueness of Mylo
The Mylo brand was created after I discovered my passion for producing herbal soaps. The goal was to make cosmetics that would gently care for the skin while pampering the senses. Since the beginning, we have produced all products in our small production unit in Bratislava, by hand, in small batches.

Target audience
The products are intended for customers who share similar values to us. At Mylo, we believe that by choosing natural ingredients produced organically, processed with respect for human rights, using environmentally-friendly, minimised packaging and eliminating waste, we as the manufacturer and our consumers can influence the future of our planet.

The most important milestone(s)
I must say, that there are several milestones. For a small, slowly developing beauty brand, hiring the first employees was a milestone in itself! Another might be the redesign of the Mylo logo and packaging in 2017 with the aim of reducing waste. Those activities brought us a nomination for the national design award. Another milestone for us was entry on the Japanese market.

Key challenge(s)
To convince and educate customers that the concept of natural cosmetics has several shades, and that the term as such can be used by brands arbitrarily and without any restrictions in the online world. That is why it is important for products to have certificates that independently confirm their claims. To convince consumers that natural cosmetics are as effective as synthetic and conventional products. To convince others that it is important to take into account the sustainable use of resources as the market for natural cosmetics grows.

Formulation work

How do you start formulating a new product?
I listen to the needs of customers and ask them what they need or what they are missing. I like to use new ingredients available on the green market when creating new formulas. I consult with books, recent studies and chemists with regard to my designed formulations. After a formula is technically developed, I ask friends or customers to test it. If the feedback is good, I prepare documentation and samples for the necessary safety report and the release of the cosmetic product on the EU market.

How many versions of your best-performing products did you make in the formulation phase?
We recently launched an eye cream product. It is performing really well so far. Before its final version, I made ten slightly different formulas of this product for tests, until I was satisfied with the results.
Is there something that you would have done differently if you were starting again?
It is hard to say, but when I think about it, if I had done something differently in the past, maybe Mylo would be a different company today, and I'm grateful for the position it's in now.

Current activities and future plans
I would like to expand my portfolio. I like to change formulas when I see the potential to improve the effect, and of course I would like to certify more products. I'm toying with the idea of replacing our solid soaps with liquid washes, the formulas of which will be in accordance with the requirements for obtaining the CosmEthically ACTIVE certificate.

CosmEthically ACTIVE certification

Where do you see the main added value for your cosmetics line? Who would you recommend the certificate to?

In this period when demand for natural cosmetics is growing and the current issue is whether natural resources are sufficient, this certificate is a very important indicator of sustainability, ethically sourced ingredients, a cruelty-free approach and the effectiveness of formulas.

What was your experience with the certification process?

I have only positive experiences, including with the approval process itself. Very professional, I must say.

About the founder

Which part/characteristic of you is imprinted in the story of Mylo?

In a few words, I would say it is respect for my fellow human beings and nature.

Who has been the most indispensable person on Mylo's journey?

That would be my partner, who has been by my side since the beginning. He supported me, encouraged me and advised me with regard to business issues, and together we came up with the Mylo brand name. The name derives from the Slovakian word for soap.

How do you find the proper work-life balance?

In the beginning, I used to work more than twelve hours a day. It made me exhausted and I started to get sick often. So, I implemented working hours from 8:00 am to 2:00 pm, and started to delegate work. I like to relax by spending time with my two kids, husband and books. I am learning to work and live according to slow living, and to capture moments that truly matter.

How do you envision cosmetics in 10 years?

I believe that we will find in stores more cosmetics that have a low negative impact on our planet during the production and consumption. More cosmetics on the market will include only active concentrations. Consumers will take care of their skin according to the slow beauty concept.

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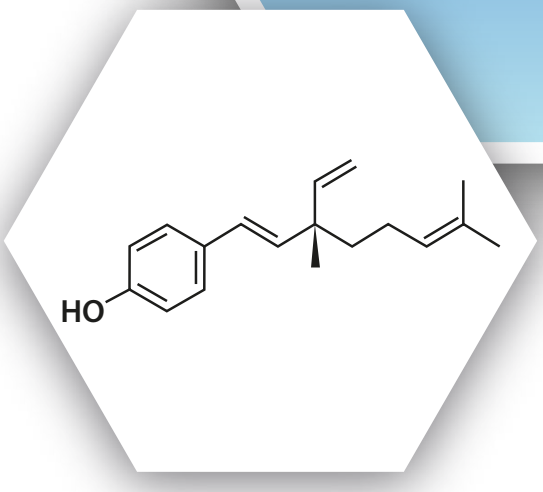

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Pura

Skincare

Welcome to the world of natural beauty.

Welcome to Pura Skincare.

Who are we?

Pura Skincare is a brand of Essentia Pura, Europe's leading manufacturer of hemp extracts, nutraceuticals and cosmetics. We are a team composed of committed, diligent, and respectful professionals, searching for the holy grail of skincare. We are passionate about medicinal plants, sustainability, and eco-industrial symbiosis and have been at the forefront of the industry since 2014. One of our goals is to create a skincare line that is not only of the highest quality, but also serves a purpose, is friendly to the environment and easy to incorporate into the daily routine.

The Mediterranean.

Our home is in the heart of the Mediterranean, where the Adriatic sea meets the Alps and where vibrant colours meet the pure simplicity of nature. Combined with the latest technological developments and using superb natural ingredients from the Mediterranean, our products are made to deliver everything your face and body need in a collection of easy-to-use, highly-effective products.

Our products & services.

Essentia Pura is one of Europe's leading CBD extract manufacturers which uses a special technique called CO2 supercritical extraction to produce the purest form of botanical extracts including those from industrial hemp. With the help of special equipment, we are capable of manufacturing various extracts in-house. The use of CO2 supercritical extraction allows us to adapt the extraction parameters individually which enables the production of highly customized extracts, unique for every client. There are many advantages of CO2 extraction compared to other, traditional techniques such as that the extract is free of impurities and contains no dangerous residues so it can be used in food products. Furthermore, the extract is antibacterial and non-toxic resulting in less post-processing steps.

The CBD extracts that we produce can be then used as a basis in food, food supplement, pharmaceutical, and cosmetic industries. Some of our best-selling products which have been proven on the market include our b2b white-label CBD Oils and our CBD Skincare line.

To learn more abut our products and services visit our website at **www.essentiapura.com.**

A brand by:

Essentia™ Pura

www.ecogea.org

Institute for quality and innovation of natural and organic products

Cosmetic Product Safety Reports

Team of experts in chemistry, pharmacy, biotechnology, medicine and toxicology with years of experience and hundreds of completed safety assessment documents.
Our ongoing training and contact with inspectors from almost all EU countries gives us valuable, up-to-date experience and an eye for detail.
Our team can help you to start selling your cosmetic products throughout Europe.

Natural and Organic Certification

Certification of natural, natural with organic portion and organic product from the field of cosmetics, cleansing products and fragrances, based on the ECOGEA standard.

Claims and Statements Verifications

CosmEthically ACTIVE certificate

New concept of cosmetics certification

CosmEthically ACTIVE is the first certificate that reviews natural cosmetics based on scientific evidence about efficacy.
It differs from all other certificates by evaluating the product's overall composition and the concentration of cosmetically active ingredients.

COSMETHICALLY ACTIVE
APPROVED BY MODERN COSMETHICS

- SCIENTIFIC EVALUATION OF A COSMETIC PRODUCT
- NATURE ABOVE ALL WHEN SELECTING INGREDIENTS
- USE OF INGREDIENTS IN COSMETICALLY ACTIVE CONCENTRATIONS
- HIGH LEVEL OF DERMAL COMPATIBILITY
- PURSUIT OF ETHICAL PRINCIPLES WITH NO ANIMAL TESTING

Scientific review

The main added value and uniqueness of the CosmEthically ACTIVE certificate is the scientific assessment of a cosmetic product during the certification process, made by independent cosmetology scientists. Scientific evaluation is a guarantee that CosmEthically ACTIVE certified products are of the highest quality and truly natural, active, skin, animal and environmentally friendly.

CosmEthically ACTIVE certified brands

MATINATA
FLOWERSPICE modern apothecary
natcosmetics
alté
mylo
MADRES
MALINCA
LUXE botanics
L'AVENTURE EN PRIMITIVANCE
JUVENTINA CLINIC
PELIA ORGANIC®
mayarula
BONISTRA Born in Istria
AVOILA
BEOTY®
L'ABEILLE
GALBAIA® NATURAL DERMATHECARY
re: SKIN ETHOS
amaranthine (adj.) undying, immortal; eternally beautiful

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MODERN COSMETICS

Ingredients of Natural Origin, A Scientific View, Volume 1

KNOWLEDGE IS THE KEY.

- The **world's most comprehensive book** about cosmetic ingredients of natural origin
- Written by **scientists**
- Sold in **93 countries**

CONTENT

482 pages, 24 chapters and 290 monographs describing **more than 500 ingredients**, including their natural sources, characteristics, the mechanism of action and use; with **rich graphic material**. Based on a review of scientific in vitro, in vivo and clinical studies.

INTENDED FOR

Professional and DIY cosmetic formulators; cosmetic scientists, pharmacists, dermatologists and students of natural sciences; natural cosmetic users.

MAIN OBJECTIVE

High-quality information about cosmetic ingredients of natural origin to raise awareness about natural cosmetics to a higher level.

BOOK REVIEWS

If this book had been around when I started out, it would have saved me about a zillion trips to the library. There are scads of books on herbs, plants, plant chemistry, uses of plants and plant-based ingredients, but Modern Cosmetics brings these components into a single, cosmetics-making context. It answers the most common questions formulators of plant-based cosmetics have when considering ingredients for a formula. This book offers something uncommon to most resource books, and that is inspiration.

Lise Andersen,
LisaLise

The information is delivered in a clear, easy-to-digest way, that appeals to a broad audience, from cosmetic formulators, researchers, industry professionals to students and DIY aficionados. Whether you're creating your own beauty products at home or simply interested in learning about key ingredients in store-bought cosmetics, this book breaks everything down simply and elegantly.

Sunny Subramanian,
Vegan Beauty Review

I would highly recommend getting yourself a copy of Modern Cosmetics if you love natural ingredients and have been looking for a solid reference text. If you're anything like me you'll find yourself reaching for it again and again – for information, for inspiration, or just to peruse and admire the beautiful pages.

Marie Rayma,
Humblebee & Me

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