KEY WORDS: COSMETIC ACTIVITY, DARUTOSIDE, ESCULOSIDE, PLANT EXTRACT, URSOLIC ACID

• *This paper discusses three new purified plant extracts and their cosmetic advantages as demonstrated by in vitro and in vivo studies. The plant extracts are darutoside, esculoside and ursolic acid.*

• *Cet article examine trois nouveaux extraits purifiés de plantes ainsi que leurs propriétés cosmétiques telles qu'elles sont révélées par des études in vitro et in vivo. Il s'agit du darutoside, de l'esculoside et de l'acide ursolique.*

• *Diese Arbeit beschreibt drei gereinigte Pflanzenextrakte und ihre Anwendungen in der Kosmetik unter Berücksichtigung von in vitro und in vivo Studien. Die Pflanzenextrakte, die beschrieben werden, sind Darutoside, Esculoside und Ursolsäure.*

•*En este trabajo se examinaron tres nuevos extractos vegetales ourificados y sus ventajas cosméticas demostradas en estudios in vitro e in vivo. Los extractos son: darutosido, esculosido y ácido ursólico.*

Purified Plant Extracts

Demonstrating the cosmetic activity of darutoside, esculoside and ursolic acid

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The use of plant extracts in cosmetic formulation is on the rise, mostly because of the poor image animal-derived extracts have acquired during the past few years. This was due, in part, to news reports regarding bovine spongiform encephalitis (BSE). Plant extracts, however, are often ill-defined as to method of extraction, plant-to-solvent ratio and the content of active ingredients. Moreover, the stability of the color, odor, transparency and/or active ingredients with time is also often a limiting factor.

Few researchers have published systematic studies of quantifiable cosmetic activity of standardized plant extracts. Many cosmetic claims are still based on traditional hearsay, folklore or obscure references. The new European cosmetic regulations, however, require more robust proof of claimed cosmetic activity. The proof depends on the two stages involved in extracting and purifying these plant materials.

First, after having obtained claim substantiation for a specific extract or mixture of extracts, we must standardize the plant collection process, the extraction method and the strict final analysis to guarantee the reproducibility of the method. The extracts obtained are usually affordable and (at times) contain a number of synergistically active ingredients. However, color, odor and stability are often difficult to guarantee over time, and the active ingredient composition may vary from harvest to harvest.

Second, we must isolate the active ingredient from the plant extract, purify it, analytically characterize it and then test it in a controlled study for cosmetic activity. This approach yields analytically well-defined products with a quality that is reproducible. Moreover, the obtained compounds are more safe due to clear dose-response relationships and toxicology and tolerance data. Most often, the powders have little odor and color, and exhibit a good stability.

This paper discusses three new purified plant extracts and their cosmetic advantages as demonstrated by in vitro and in vivo studies. The plant extracts are darutoside, esculoside and ursolic acid.

Darutoside

Darutoside (Figure 1-1) is a trihydroxy-diterpene molecule known to stimulate wound healing and tissue regeneration by way of collagen matrix build-up.

This paper was presented at the Active Ingredients Conference in Paris on November 12, 1997, and published in the proceedings.

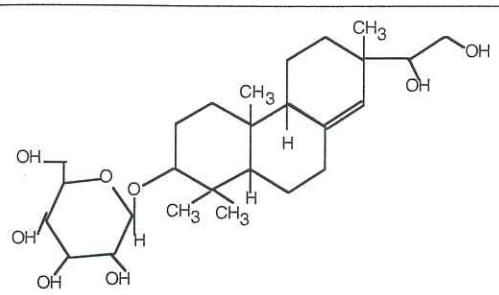

Figure 1-1. Structure of darutoside

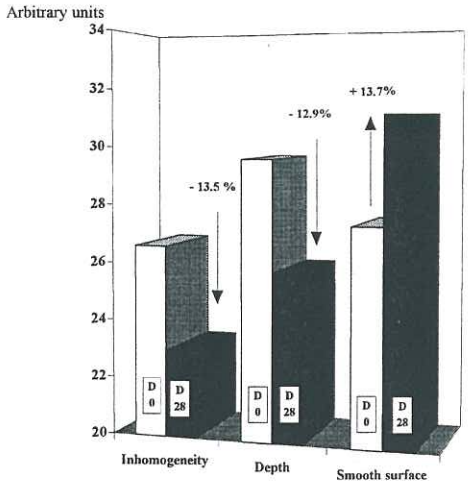

Figure 1-2. Stretch mark characteristics before and after four weeks of treatment with darutoside

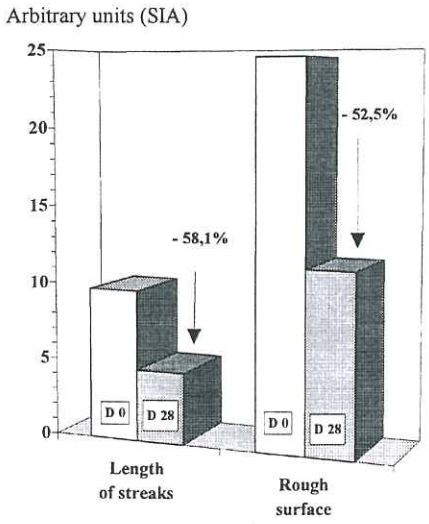

Figure 1-3. Stretch mark characteristics before and after four weeks of treatment with darutoside

The most detailed description of darutoside appears in a now-expired patent by Pierre Fabre Medicaments.[11] The authors show by histological and histochemical methods that darutoside stimulates wound healing. It leads to more regular tissue renewal, to normal appearance of the scars and to full restoration of elasticity. Collagen fibers are then arranged in regular fashion.

The trihydroxy-diterpene structure of darutoside can be compared to the triterpene structure of asiatic acid extracts from *Centella asiatica*. Its structural similarity might explain its similar properties for the collagen synthesis stimulation, matrix regeneration and wound-healing activity.

Darutoside-enriched total extracts of *Siegesbeckia orientalis* have been shown to possess in vitro and in vivo anti-inflammatory properties (inhibition of lipoxygenase pathway of prostaglandin synthesis, inhibition of collagenase, protection against UV-induced erythema). *Siegesbeckia orientalis* is the primary source of darutoside. The plant is native in Ethiopia, but mainly found in Madagascar, where it is called Satrike Aza Marartra. It also grows in Japan, Australia and the Dekkan peninsula. In Reunion and Mauritius islands, it is known under several names referring to its healing properties: divine grass and guerit-vite (heals rapidly). Popular uses of the plant are external wound healing and soothing of inflammation.[11]

One obtains pure darutoside by fractionated recrystallization of the ethanolic extract from the plant *Siegesbeckia orientalis* L.

Anti-Stretch Mark Activity of Darutoside

The efficacy of darutoside on the matrix of the dermis led us to an in vivo test of its potential in restructuring stretch marks. Stretch marks consist of surface damage to the skin. This damage usually results from abnormal stretching of the dermis during pregnancy or weight change, from a linear scar or from certain endocrine disorders.

The histopathology of stretch marks shows that these lesions affect the elastin fibers in the middle dermis, causing them to diminish or disappear. The collagen fibers may also be affected. The accumulation of abnormally large quantities of granulo-filamentous interfibrillar material and the abnormalities of the elastin fibers and of collagen indicate abnormal polymerization of these fibers, which can be seen under electron microscopy.

The cosmetic treatment of stretch marks, therefore, involves reducing this filamentous material and restoring normal quantities of collagen fibers and elastin microfilaments and better dermo-epidermal cohesion. Stretch marks are usually clearly visible and sometimes even palpable on the surface of the skin, so the efficacy of treatment intended to remedy them can be investigated using the technique of printing and surface analysis.

An in vivo test: The test involved an evaluation of the anti-stretch mark efficacy of a cream containing 1.5% of a 1.0% solution of darutoside. We obtained skin replicas before and after treatment lasting four weeks in adult female volunteers presenting recent or long-established stretch marks. The replicas of the zones affected were used to provide an objective evaluation of any change in the relief of the skin.

The panel taking part in the study consisted of 14 adult female volunteers between the ages of 21 and 42. All had stretch marks on the abdomen, thighs, hips, buttocks or breasts. The volunteers reported that the stretch marks had resulted from one or more pregnancies, from fluctuations in bodyweight or, in one case, from rapid growth.

Each volunteer applied the cream twice daily for four consecutive weeks.

Before the study began, each volunteer rested for 15 min lying down with the clothing removed from the area of the body affected by the stretch marks so that any marks

produced by the clothing would disappear. The subjects were examined on Day 0 and Day 2, at the same time of day, having not applied any cosmetic product since the previous evening.

The experimental determinations were carried out under specific environmental conditions of temperature and relative humidity, which were checked for each volunteer.

We took replicas[a] and subjected them to image analysis using appropriate software. We calculated the mean values from four different stretch marks. Then we compared the biometric parameters obtained before and after treatment. We also analyzed the subjective assessments of the panel subjects.

Results: After the end of treatment, the surface of the skin was more regular in appearance, the global profile was more uniform and the stretch marks were tending to fade. Figures 1-2 and 1-3 summarize the quantitative results.

Seven of the 14 volunteers assessed the treatment as being effective at reducing the depth and size of the stretch marks, and also at reducing their sensitivity to touch. One volunteer saw no effect at all and the others expressed no opinion. In some cases, the efficacy of the treatment was assessed as very definite and visually discernible.

Cosmetic implications: Published reports indicate excellent tolerance and non-toxicity for darutoside.[11] Our in vivo anti-stretch mark study suggests use of this natural molecule in the following specific cosmetic treatments: firming (collagen matrix renewal), anti-stretch mark and antiaging.

Our in vivo study substantiates its activity in a use concentration of 1.5% of a 1.0% solution.

Esculoside

Esculoside (Figure 1-4) is listed in the ninth edition of the French pharmacopoeia. Esculoside-containing pharmaceutical specialties and folk medicinal remedies abound.

Bark of horse chestnut branches *Aesculus hippocastanum L.* is the primary source of esculoside. Alcoholic or aqueous extracts of the bark are described as veinotonic, antipyretic and congestion-relief preparations for internal or external use.[2,3,12,19]

Esculoside is commonly used to treat red skin blotches, heavy legs and edemas,[14,15] as well as to increase the capillary resistance (vitamin P properties), decrease capillary permeability and participate in anti-inflammatory activities.[17]

Marinova et al described the antioxidant activity of esculetin (esculoside without the sugar moiety) as manifested by protection of triglycerides against auto-oxidation at high temperatures.[9] This antioxidant property might explain some of the anti-inflammatory activities of esculoside.

Yamagami et al showed reduction of carageenin-induced edema and UV-related erythema, as well as diuretic effects.

The cosmetic applications of horse chestnut extracts include local hyperhemia treatment (sun exposure), the reduction of irritation and skin rashes, and the increase of local microcirculation in association with lipolytic slimming, anti-cellulite treatment.

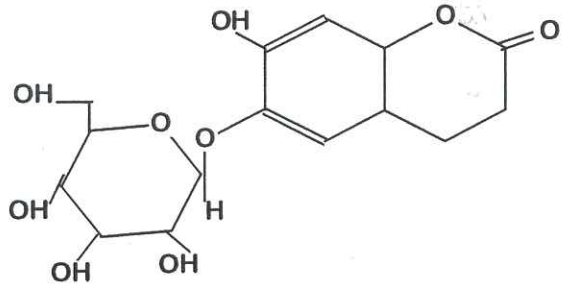

Figure 1-4. Structure of esculoside

Huei-Chan Huange et al studied antiproliferative effects of esculoside and esculetin and showed inhibitory activity on protein tyrosine kinase.[4]

Kostova et al described anti-microbial activity of esculoside and esculetin as well as skin regeneration after wounding.[6] They also confirmed the absence of irritation or toxicity from esculoside.[5]

One obtains pure esculoside by extraction from *Aesculus hippocastanum* L. with hot water, followed by precipitation of the tannins and fractionated recrystalization with active carbon filtration steps.

Anti-Couperose Activity of Esculoside

The veinotonic and anti-inflammatory properties of *Aesculus hippocastanum* L. led us to an in vivo test of its cosmetic action in treating blotches. Blotches consist initially of a symmetrical inflammation of the face (transient erythema) following stress, changes in temperature or alcohol consumption. This inflammation may become persistent (couperose) and give rise to the telangiectasis known as facial blotchiness. We do not know the precise cause of these symptoms, but their onset and progress are probably determined by microcirculatory vascular disturbances in the facial angular veins, which are directly involved in cooling the brain.

In severe cases of blotchiness, the recommended treatments consist of electrosurgery, sclerotherapy, CO_2 laser therapy or chemical peeling. The painless and non-aggressive cosmetic treatment consists of the application of active substances containing molecules that affect the tone of the veins and venules in the surface of the skin and strengthen the vessel walls, facilitating the flow of blood.

An in vivo test: The in vivo test used to detect anti-couperose activity is based on measuring the superficial blood flow[b] before and after prolonged treatment of one side of the face. The measurement conditions are those recommended by Tenland[16] and Bircher et al.[1]

We selected 13 female volunteers between the ages of 20 and 60 (group mean: 40±14 years) who presented obvious signs of blotchiness. They were recruited on the basis of a health questionnaire plus the usual inclusion and exclusion criteria.

A clinical examination of the severity of the blotchiness (scored from 0 to 4) on the cheeks made it possible to identify two volunteers who presented with mild blotchiness (redness), five with marked blotchiness (severe redness),

[a] Silflo, Flexico Developments Ltd, Potters Bar, England

[b] Laser-Doppler Flow meter, Periflux pf ld, Perimed KB, Stockholm, Sweden

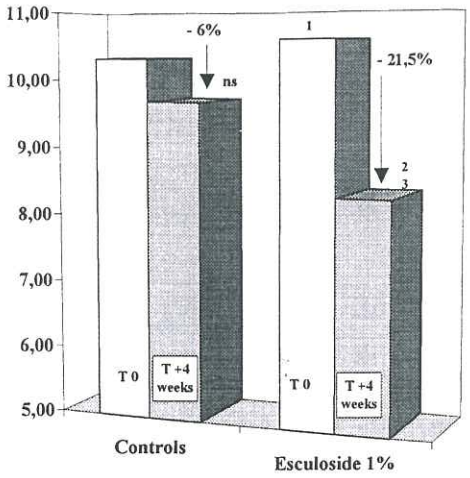

Figure 1-5. Anti-couperose activity of esculoside, as indicated by flowmeter measurements of cutaneous microcirculation before and after four weeks of treatment, compared to treatment with a control

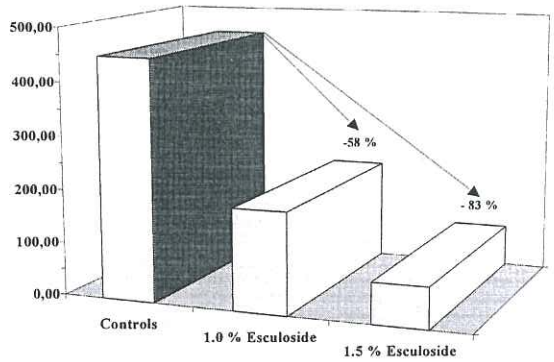

Figure 1-6. Free radical scavenger activity of esculoside, as indicated by optical density measurements

four with very marked blotchiness (severe redness and marked vascularization) and two with severe blotchiness (severe redness and very marked vascularization).

Protocol: On Day 0 (at the beginning of the trial) we allowed each volunteer to rest for 20 min. We monitored the temperature and relative humidity conditions for each individual. Determinations were carried out in a calm atmosphere with no auditory or visual stimuli. With the flow meter, we measured the microcirculation in both cheeks of each volunteer: one cheek was used as the control site and the other as the treated site.

We examined the measurement zones using lenses specially designed for this purpose. We took repeated measurements from each site and reported the mean value.

The volunteers applied an emulsion containing 1% of esculoside twice daily for four weeks to the right side of their face, the other side serving as the untreated control.

On Day 28, we followed the same operating procedures for the biometric determinations. We expressed the study data in unitless microcirculation values, which were read from the Lineais recorder plot.

Results: Figure 1-5 summarizes the differences (means) in blotchiness (telangiectases) resulting from treating the skin of the face with 1% esculoside.

Flow meter measurements indicated that esculoside reduced the cutaneous microcirculation by 21.5%. Statistical analysis (Student's t-test for paired values) showed that this reduction was significant, whereas any changes found during the same period for the untreated side were minor and not statistically significant.

Cosmetic implications: Results of our in vivo study suggest esculoside's use in the treatment of red blotches of the skin and couperose.

Free Radical Scavenger Activity of Esculoside

An in vivo test: In this study we applied emulsions containing various concentrations of esculoside to the skin, waited 15-30 min until the product had all penetrated and been distributed, and then removed the corneal layer by the stripping method. We exposed the adhering sample obtained in this manner to 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution for 30 min. Then we read the optical density (OD) on a UV-vis spectrophotometer[c] at 517 nm to determine the degree of inactivation of the free radical. This makes it possible to determine the persistance of the effect of the active principle in the skin.

Results: Figure 1-6 shows the values of the OD and the percentage inactivation of the DPPH radical after 30 min of exposing the esculoside-treated skin sample to DPPH solution. The figure shows that the effect is dose-dependent and that the activity detected in vitro can be reproduced ex vivo. At an esculoside concentration of 1.5%, approximately 82% of the free radicals were inactivated.

Ursolic Acid

Ursolic acid (Figure 1-7) is primarily a powerful natural anti-inflammatory agent, but it also possesses other activities. Several in vitro studies have shown its capacity to inhibit certain enzymes that are held responsible for inflammatory reactions.

- Lipoxygenase and cyclooxygenase. Lipoxygenase and cyclooxygenase are enzymes that metabolize arachidonic acid into leukotrienes and prostaglandines, mediators that initiate the inflammatory cascade (infiltration, edema, degranulation of mastocytes). Adding ursolic acid in vitro into the culture medium of different cell populations (macrophages, platelets, granulocytes) leads to an inhibition of the enzymatic activities on the order of 40-85%.[10]
- Elastase. Human leucocyte elastase is a lysosomal proteinase that is storied in large quantities in polymorphonuclear leucocytes involved in the destruction of tissue during inflammatory reaction to chemical or mechanical aggression. Ursolic acid is one of the most powerful inhibitors of this proteolysis.[22]It apparently is able to bind directly to the enzyme by way of electrostatic bonds between the carboxyl group and the active site of the elastase. Cosmetics researchers are seeking elastase inhibitors to help in the fight against aging, whose symptoms (wrinkles, flaccid tissue) are at least partly due to progressive elastolysis of the elastic fibers in the dermis. This elastolysis is also stimulated by excessive solar irradiation.

[c]Uvikon 922, Kontron Instruments, Montigny, France

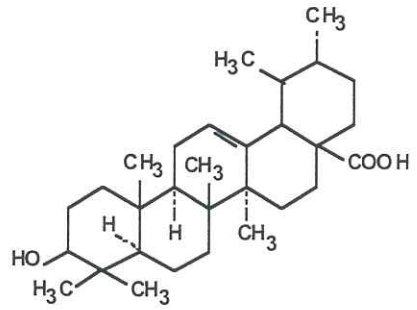

Figure 1-7. Structure of ursolic acid

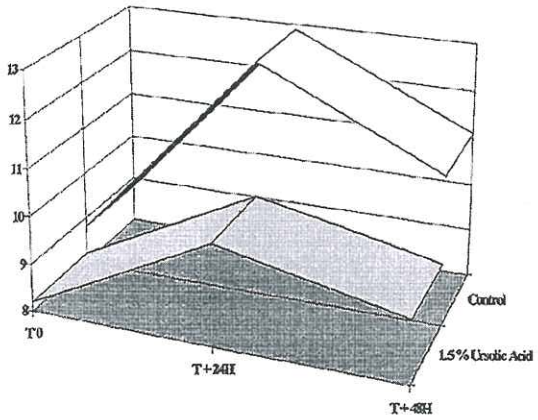

Figure 1-8. Effect of ursolic acid in reducing cutaneous redness

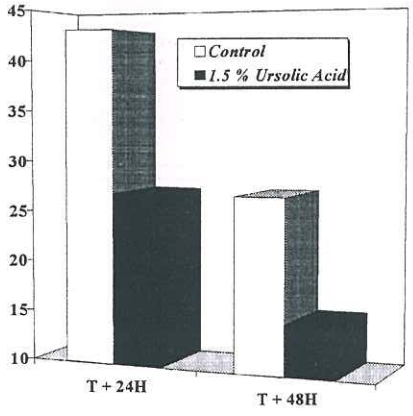

Figure 1-9. Percentage change in erythema values as a result of treating affected skin with ursolic acid versus treating with a control

- Histamine release from mastocytes. Histamine contained in mastocytes are very powerful mediators of inflammation and allergy. That explains their pharmaceutical use in anti-histaminic drugs. An in vitro study by Tsuruga et al showed that ursolic acid extracted from *Melaleuca leucodendron* strongly inhibits the histamine release from mastocytes when they are induced into degranulation by activators, such as concanavaline A.[18]

The anti-inflammatory activity tests described in earlier literature deal with in vivo studies on animals. Examples include inhibition of edema in rats paw caused by injection of carraghenin (standard test of anti-inflammatory activity). Three publications mention ursolic acid as a very powerful active ingredient in these tests.[21,8,7]

Rosemary (*Rosemarinum officinalis* L.) is the primary source of ursolic acid. Pure ursolic acid is obtained by fractionated recrystallization of the ethanolic extract of rosemary.

Anti-inflammatory Activity of Ursolic Acid

An in vivo test: To confirm the anti-inflammatory activity of ursolic acid for cosmetic purposes, we carried out an in vivo test in a panel of volunteers at an off-site clinic.[d] The panel consisted of ten volunteers (three men and seven women) whose ages were between 22 and 32.

Because the irritant potential of sodium lauryl sulfate (SLS) has been well documented, we used it to provoke skin irritation and then applied a lotion containing 1.5% ursolic acid to determine its soothing effects.

Protocol: Before treatment, clinic technicians measured[e] the color of the skin of two ringed zones on the volar forearm of each volunteer, and then applied a dilute solution of SLS under an occlusive patch for 24 h. Immediately afterward, and then 12 and 24 h later, the technicians applied 1.5% (w/w) ursolic acid lotion to one of these two zones, leaving the other untreated as the control zone.

At 24 h and again at 48 h after removing the patch, technicians measured the color of the skin (and the severity of the erythematous reaction) in the two zones. They recorded the "a*" values (redness) for analysis. We subjected the raw data, means and standard deviations to statistical analysis (Student's t-test).

Results: The lotion containing 1.5% ursolic acid reduced the erythematous reactions induced by SLS (Figure 1-8). Statistical analysis showed that skin redness increased markedly after the SLS treatment ($p<0.05$). However, the increase in redness was definitely less marked after treatment with the lotion both after 24 h and after 48 h (Figure 1-9). The differences between the raw data and the baseline values were significant ($p<0.05$).

Cosmetic implications: Researchers have documented excellent tolerance and absence of toxicity for ursolic acid.[13] Our in vivo study of its anti-inflammatory activity suggests the use of this natural molecule for specific cosmetic applications in smoothing sensitive skins, in caring for diffuse redness, and in products for sun and after-sun use. Our study also substantiated the activity of ursolic acid when used at a concentration of 1.5%.

Conclusion

New trends in legislation, cosmetic science, consumer awareness and expectations require new solutions to old problems. Some animal-derived products need to be replaced, but synthetic chemicals cannot always do this well. Natural molecules derived from plant extracts offer a particularly exciting venue for further research because the richness of the plant kingdom has only begun to yield its secrets.

This approach, however, requires the multidisciplinary cooperation of botanists, preparative chemists, analytical chemists, toxicologists and biologists to assess cosmetic,

[d] Institut d'Expertise Clinique, Lyon, France

[e] CR 300 Chromameter, Minolta, Osaka, Japan

rather than just pharmaceutical, activity. We are working on extending this research program to further new, fully characterized molecules of herbal origin for various cosmetic applications.

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