Argassential™
by Beauty Creations

From argan pulp to naturally plump and densify the skin

Scientific brochure
Argassential™  |  BC09959
--- | ---
**Origin - Description** | Argan pulp supercritical extract
**Regulatory data** |  
**INCI** | Dicaprylyl Ether (and) Sorbitol (and) Lauryl Glucoside (and) Polyglyceryl-2 Dipolyhydroxystearate (and) Water (and) Argania Spinosa Fruit Extract (and) Glycerin
**China** | Each component listed in Inventory of Existing Cosmetic Ingredient in China (2014) IECIC Argania Spinosa Fruit Extract listed as Argania Spinosa Extract in IECIC
**Appearance** | Yellow-orangey to amber viscous liquid
**Preservative** | None
**Natural labels** | Raw material conform to Ecocert and Cosmos standard of Natural and Organic Cosmetics
**Cosmetic use** |  
**Properties** | • Boosts type I collagen to densify the dermis and firm the skin  
• Fights against adipocytes ageing by increasing pre-adipocytes differentiation & lipid storage to plump the skin  
• Improves firmness and elasticity  
• Plumps the cheeks  
• Plumps the lips  
• Daily anti-ageing treatment  
• For skin and lips
**Formulation data** |  
**Concentration of use** | 1 to 2%
**Solubility** | Hydrodispersible & lipodispersible
**Incorporation method** | Argassential™ BC09959 is incorporated into the finished process below 40°C, or at room temperature for cold processing
**Optimal pH** | 3 - 9
**Storage** | In its original packaging and protected from light and heat, at 10-30°C
**Shelf life** | 12 months

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Volume and density keep you looking young

**What happens with age?**

With intrinsic ageing, there is a loss of skin volume and density (fig. 1). Two phenomena are involved in this cutaneous loss:

- **the degradation of the dermis**, particularly the thinning of the dermis and loss of the components of the extracellular matrix (ECM). The type I collagen represents one of the major connective materials synthesized by dermal fibroblasts and is the predominant collagen in human dermis [1]. Collagen fibers provide strength to the dermis and support to human skin [2]. Their principal function is to maintain skin firmness. It is well established that the amount of collagen type I decreases in chronically aged as well as in photo-aged skin. This collagen decrease is the result of an enhanced degradation by proteinase but also of a lowered synthesis by dermal fibroblasts [3, 4].

- **the lipoatrophy**, a fat loss or adipose loss. With age the fat cells function diminishes, thus leading to a decrease of their size and an impaired differentiation. The most noticeable signs of the onset of lipoatrophy occur on the face.

**Lipoatrophy with age**

With intrinsic ageing, there is a loss of skin volume and density (fig. 1). Two phenomena are involved in this cutaneous loss:

The ageing of the face is characterized by a decrease of volume, mainly around the eyes and cheeks which is associated with the sagging of skin and a loss of the oval of the face. Among the major causes contributing to facial ageing there is a subcutaneous fat loss and redistribution [5]. To obtain youthful rejuvenation, a surgical rebalance of fat distribution has been proposed [6]. In fact, this disappearance of subcutaneous adipose tissue is linked to the ageing of adipocytes. It is established that the capacity of pre-adipocytes to proliferate and to form fully functional adipocytes declines with age [7]. Therefore, besides surgical interventions, an alternative way for facial rejuvenation could be to fight against adipocyte ageing by improving their differentiation process.

**Two-dimensional approach to facial rejuvenation**

Besides surgical interventions, an alternative way for facial rejuvenation could be a two-dimensional approach:

- densify the dermis by enhancement of collagen type I synthesis,
- fight against “adipocyte ageing”, i.e. rejuvenate adipocytes by increasing differentiation and lipid storage.

Visible consequences will be an increased volume and density of the skin that looks younger.
Argassential™: new ingredient from our Argan program to naturally plump and densify the skin

Safety / Tolerability of the product
Argassential™, an argan pulp extract obtained through supercritical CO₂/EtOH extraction, was assessed to ensure its safety in the recommended conditions of use. Argassential™, is not irritating to the eyes and skin under conditions of use. No indication of skin sensitization was observed.

Description of the plant
Family: Sapotaceae  
Species: Argania spinosa (L.) Skeels  
Common name: argan.
Distribution: the argan tree is indigenous to Southern Morocco. It is largely found in the South-East of Essaouira on the Souss plain. The argan forest extends over approximately 800,000 hectares and contains more than 20 million trees. It acts as a natural barrier against the progress of the neighboring Sahara desert. The argan fruit contains a very hard shell holding between one and three kernels, from which a valuable oil can be extracted. The argan forest is extremely important in socio-economical terms, as it supports over three million people (out of a total Moroccan population of just over 30 million).
Description: the tree, particularly resistant to dry and arid conditions of this region, can resist to temperatures ranging from 3 to 50°C and grows at altitudes of up to 1500 meters. Its very deep roots extend over a large area and can search out water at more than 30 meters under the ground. This long-life tree (150 to 200 years) reaches heights of 8 to 10 meters and has a shape similar to an olive tree. Argan leaves are small and dark green. The tree has small oval greenish yellow fruits which become brown when they ripen and contain a very hard shell enclosing one to three almond like kernels.
Plant part used: pulp, obtained from the fruit.

Sustainable sourcing of argan pulp is ensured through a long term partnership with local women cooperatives in Morocco
A fragile natural resource from Southern Morocco
Unfortunately, due to demographic pressure, argan woodland suffers from continuous degradation. Over-exploitation, soil erosion and advancing desertification are amongst the threats to this unique heritage. Indeed, the climate, which is always hard in these regions, is accompanied by erosion making the soil even more arid. Excessive grazing makes it more difficult for the argan trees to grow properly. Moreover, the growing Moroccan population leads to an increase in wood consumption, which is used both for construction and as firewood.
**Description**

*Argassential™* is a preservative-free argan pulp extract, obtained through a supercritical CO₂/EtOH extraction process.

*Illustration of a commercial sample of Argassential™ 8039959 with an emulsion containing 2%.*

**Responsible valorization**

The valorization of the argan pulp is part of the BASF’s Argan program. In this framework a responsible partnership has been implemented since 2005 with Targanine network of women cooperatives in Morocco (please consult Argan Program Brochure).

**Product processing**

**The raw material: argan pulp**

The fruits are collected on the ground and de-pulped in female cooperatives according sustainable social guidelines. The pulp is thus obtained as a by-product of the argan oil production. Approximately, 10 tons of pulp are generated by the production of 1 ton of argan oil. This raw material is organic certified.

**Processing the pulp to obtain Argania Spinosa Fruit Extract**

Argania Spinosa Fruit Extract is obtained by supercritical CO₂/EtOH extraction of argan pulp.

**From Argania Spinosa Fruit Extract to Argassential™**

Argassential™ is obtained by mixing the active matter (Argania Spinosa Fruit Extract) at 10% (w/w) with adjuvants, in order to obtain a hydrodispersible and lipodispersible cosmetic ingredient.

**Phytochemistry**

Argania Spinosa Fruit Extract is rich in esterified triterpenes, in particular derivatives of lupeol, α- and β-amyrin. This raw material is organic certified.
With intrinsic ageing, there is a loss of skin volume and density.

The active matter of Argassential™, Argania Spinosa Fruit Extract, was first evaluated *in vitro* by a two-dimensional approach:
- evaluation on human skin fibroblasts in culture, of its efficiency to enhance type I collagen (one of the main collagen of the dermis) synthesis to densify the dermis,
- evaluation of its capacity to fight against “adipocyte ageing”, *i.e.* to rejuvenate the adipocytes by increasing the pre-adipocytes differentiation and lipid storage.

*In vivo*, Argassential™ was evaluated on its ability to firm and plump the skin and the lips for a rejuvenation of facial appearance.
Effect of Argania Spinosa Fruit Extract on type I collagen (in vitro tests)

OBJECTIVE
The purpose of this study was to demonstrate via ELISA test and immunocytochemistry the ability of Argania Spinosa Fruit Extract to increase type I collagen synthesis.

Type I is composed of long protein chains wrapped in a triple helical structure which is produced and secreted by fibroblasts as a procollagen form. Extracellular procollagen is converted in collagen by proteinases that remove terminal peptides and then collagen molecules are structured by stable crosslinking into fibrils and then fibers, which provide strength to dermis and firmness to human skin.

The amount of collagen type I decreases in chronically aged as well as in photo-aged skin, in particular because of a lowered synthesis by dermal fibroblasts with age [4]. So, improving type I collagen synthesis is a good way to fight against the age-related loss of skin firmness and density.

RESULTS & DISCUSSION

ELISA TEST
TGF-β1 at 3 ng/ml (used as positive control) significantly increased the level of cellular proteins and it enhanced the amount of released type I procollagen and cellular proteins from human dermal fibroblasts (fig. 3).

Argania Spinosa Fruit Extract at 0.00125% - 0.005% moderately increased the level of cellular proteins and it strongly enhanced the amount of released type I procollagen from human dermal fibroblasts, up to 73% (fig. 3).

IMMUNOCYTOCHEMISTRY
The synthesis of the type I collagen by fibroblasts was evaluated by immunostaining; the presence of type I collagen in the culture was visualized by a green fluorescence of the FITC (fig. 4) and quantified as the % of fluorescent area in the culture (fig. 5).

A low level of type I collagen was detected in the fibroblasts cultured without treatment (control). Addition of TGF-β1 at 10 ng/ml (used as positive control) strongly enhanced the synthesis of type I collagen by the fibroblasts.

The addition of Argania Spinosa Fruit Extract at 0.003% and 0.006% in the culture clearly increased (x 8.7) the synthesis of type I collagen in the human fibroblasts in culture compared to untreated control culture (fig 4 and 5).
MATERIALS & METHODS

Cell culture
Human dermal fibroblasts were prepared from adult human skin (donors from 33 to 51 years old) and cultured to cells confluence in standard growth medium containing foetal calf serum (FCS) at 37°C with CO₂ at 5% and 95% of humidity.

Treatment
After cells confluence, the culture medium was exchanged for standard medium and 3 conditions were performed:
- Control: standard medium without product added
- Positive control: addition of TGF-β₁
- Argania Spinosa Fruit Extract addition at different concentrations.

ELISA test
After 3 days of incubation at 37°C with CO₂ at 5% and 95% relative humidity, collagen synthesis was evaluated by measure of the amount of released Carboxyl (C) terminal peptide from treated fibroblasts [8]. For that an aliquot of supernatant medium was recovered and C terminal peptide release was determined by an ELISA method according to the recommendation of provider. Cell layers were rinsed and homogenized in NaOH and the amount of cell proteins was determined on cell homogenate by Bradford's method.

Immunocytochemistry test
After 2 days of incubation at 37°C, 95% relative humidity and 5% CO₂, the culture medium was removed and cells were quickly rinsed with PBS. Then, cells were fixed by acetone for 10 minutes at -20°C. After rinsing, cells were incubated at 37°C with the primary antibody anti-collagen I at room temperature. After 1 hour of incubation, cells were rinsed and incubated for 45 minutes at room temperature with the secondary antibody labelled with FITC. Finally, cells were rinsed with PBS and counterstained by blue Evans before observation by confocal microscopy.

Results and statistics
For ELISA test, the results were calculated in % referring to the control (standard medium without addition of ingredients) for each assay and then expressed as a mean ± SD (standard deviation) from the 2 assays in triplicate. The product and reference effects were statistically evaluated by the Student’s t test.
For immunocytochemistry test, the cells were viewed by confocal laser scanning microscope and 6 pictures were done for each condition. The immunostaining was quantified by image analyser and expressed in percentage of occupation of type I collagen in the culture. Average results were obtained by pooling the results obtained with 4 independent assays. The product and reference effects were statistically evaluated by U test of Mann and Whitney.

CONCLUSION
We have demonstrated that Argassential™ significantly stimulates type I collagen synthesis from human dermal fibroblasts, thus Argassential™ can be used to redensify the aged dermis.
Effect of Argania Spinosa Fruit Extract on human adipocytes (*in vitro* tests)

**OBJECTIVE**
The purpose of this study was to demonstrate the ability of Argania Spinosa Fruit Extract:
- to stimulate pre-adipocyte differentiation,
- to increase lipid storage in mature adipocytes.

Adipogenesis refers to the process of adipose tissue generation which involves two key steps: first, the proliferation and differentiation of precursor cells into functional differentiated pre-adipocytes. Then these differentiated pre-adipocytes accumulate triglycerides in a large cytoplasmic vacuole of lipids which characterizes the mature adipocytes (fig. 6). This process occurs throughout life and assumes the regeneration of adipose tissue [9].

**RESULTS & DISCUSSION**

**ACTIVATION OF PRE-ADIPOCYTES DIFFERENTIATION**
To evaluate the effect of Argania Spinosa Fruit Extract on the differentiation of precursor cells into functional pre-adipocytes, it was applied only during this first differentiation phase (fig 7). These functional pre-adipocytes were then cultivated within standard medium up to the formation of mature adipocytes with large lipid droplets.

Argania Spinosa Fruit Extract at 0.01% has increased the differentiation of human pre-adipocytes, leading to +69% of lipids in the mature adipocytes cells (fig. 8).

In parallel, no cytotoxicity was observed. Argania Spinosa Fruit Extract at 0.01% has moderately increased the cell growth characterized by cell DNA level (+8%) (fig. 8).

![Figure 6: The main steps of adipogenesis.](image)

![Figure 7: Activation of differentiation into functional pre-adipocytes.](image)

![Figure 8: Lipids and DNA levels after treatment with Argania Spinosa Fruit Extract during the differentiation phase of undifferentiated pre-adipocytes into functional pre-adipocytes.](image)
After the phase of differentiation into functional pre-adipocytes, the phase of maturation begins with accumulation of lipids in large droplets. To evaluate its potential on adipogenesis, Argania Spinosa Fruit Extract was applied during both differentiation and maturation phases (fig. 9). Argania Spinosa Fruit Extract at 0.005% and 0.01% improved the adipogenesis in a dose dependent manner. A significant increase of respectively +71% and +325% of lipids accumulation was observed (fig. 10).

In parallel, no cytotoxicity was observed. Argania Spinosa Fruit Extract at 0.01% has moderately increased the cell growth characterized by cell DNA level (+14%) (fig 10).

CONCLUSION

We demonstrated with Argassential™ a significant increase of the differentiation of human pre-adipocytes and an increase of lipids storage in mature adipocytes up to 325%. Argassential™ stimulates significantly human adipogenesis and therefore can be used to fight against cellular lipotrophy.

Figure 9: Activation of differentiation and maturation for lipid storage.

Figure 10: Lipids and DNA levels after obtained after treatment with Argania Spinosa Fruit Extract during the differentiation and maturation phases.

MATERIALS & METHODS

Cell culture

Undifferentiated human subcutaneous pre-adipocytes were seeded in growth medium cells for 2 to 3 days. To mimic the adipogenesis process, a culture involving two sequential phases has been used:

- differentiation phase: induction of differentiation into functional pre-adipocytes by differentiation medium containing insulin, dexamethasone and isobutylmethylxanthine during 3 days,
- then a maturation phase: final maturation with lipid accumulation by incubating adipocytes in differentiation medium containing foetal bovine serum during around 8 days. The differentiation medium is renewed three times a week.

Treatment

To study the influence on differentiation into functional pre-adipocytes, Argania Spinosa Fruit Extract was included in the culture medium only during the differentiation phase, but not during the maturation phase.

To study adipogenesis (differentiation, then lipids accumulation), Argania Spinosa Fruit Extract was added during both the differentiation and maturation phases. [10, 11].

Assay

After both differentiation and maturation phases, cell layers are rinsed by PBS and then fixed by a formaldehyde solution. Lipids are stained by Nile red reagent while cell DNA was stained by Hoechst reagent. The amount of lipids is measured by recording the fluorescence of Nile red at 625 nm (excitation at 520 nm) while DNA level is measured by recording the fluorescence of Hoechst reagent at 465 nm (excitation at 356 nm).

Results and statistics

The results were calculated in percentage referring to the control (standard medium without addition of ingredients) for each assay and expressed as a mean ± standard deviation (SD) from assays in triplicate. The product effects were statistically evaluated by the Student’s t test.
OBJECTIVE

Visible and measurable effects of Argassential™ on the skin were assessed by 3 placebo-controlled clinical studies on human female Caucasian volunteers.

Ageing causes structural and molecular alterations of the biomechanical properties of the skin leading to a loss of skin firmness and elasticity, characterized respectively by an age-related decrease of Cutometer® parameters R7, Q1, Q3 and R2, R5 [12]. The first test was conducted on female volunteers to appraise the effect of Argassential™ on the biomechanical properties of the skin on the face (fig. 11).

The most noticeable signs of the onset of age-related lipoatrophy (adipose loss) occur in the face. There is loss of volume in the buccal fat pads and throughout the dermal layers leading to sunken cheeks and temples, accentuated facial folds with shadowing, and protruding facial musculature and bony landmarks. The second test was carried out on female volunteers to study the plumping effect of Argassential™ on the face (fig. 12). Oblique face profiles were obtained by drawing two lines on cheekbones, and superposed to measure the difference between D0 and D56.

The lips of the young woman are full bodied and smooth. During ageing, the lips begin to flatten and thin out, the corners droop, and the lip lines begin to form. The third test was carried out on female volunteers to evaluate the plumping effect of Argassential™ on the lips (fig. 13). The volume of the lips was evaluated by fringe projection at D0 and D28.
RESULTS & DISCUSSION

FIRMING EFFECT ON THE FACE
Application of placebo formulation lead to a decrease of all studied Cutometer® parameters. Comparatively, after treatment with the same formulation containing Argassential™ at 2%, we observed a significant 13.1% increase of the parameters R5 (p<0.05) (fig. 14). Additionally, the 8.6% increase vs placebo of R2 parameter is close to significance (p<0.1). These increases correspond to an improvement of skin elasticity.

Comparatively to placebo, Argassential™ at 2% increased also the parameters R7 by 13.3% and Q1 by 10.0% (p<0.05) (fig. 14). Additionally, we observed a 7.7% increase of Q3 parameter, even if not significant. These increases correspond to an improvement of skin firmness.

Argassential™ at 2% significantly improved skin biomechanical properties vs placebo: it outperformed the placebo significantly by increasing the elasticity by 13.1% (R5), and the firmness by 13.3% and 10% (R7 and Q1).

PLUMPLING EFFECT ON THE FACE
After 56 days of treatment on female volunteers with the formulation containing Argassential™ at 2%, we have observed a positive difference in the lateral profile of cheekbones (fig. 15). This difference has a limited significance (p<0.1) comparatively to both baseline (D0) and placebo (D56), whereas placebo variation versus D0 was not significant. This shows that the emulsion containing Argassential™ at 2% has plumped the face skin (with 90% certainty).

PLUMPLING EFFECT ON THE LIPS
After 28 days of application, the lipstick containing Argassential™ at 2% increased significantly the volume of lips by 8.4% versus baseline (D0) whereas the placebo lipstick had no significant effect (fig. 16).

CONCLUSION
The formulations containing Argassential™ have improved the skin’s firmness and elasticity significantly comparatively to placebo, while increasing the volume of the lips and plumping the cheeks. Argassential™ can be used to improve the youthful appearance of the skin, to plump and densify the skin and lips by a non-invasive procedure.
Study on the face of the firming effect – Evaluation of biomechanical properties by Cutometer®

Study design
The clinical study was carried-out as a placebo-controlled double-blind randomized split-face study. The formulations are detailed in Annex 2. The efficacy of the formulation containing Argassential™ at 2% was compared to the baseline (before treatment, D0) and to the other half-face treated with placebo. The study was conducted during a period of 56 days with check points at D0 and D56. This study was carried out from October to November.

Inclusion criteria
The tests were done on 29 female Caucasian volunteers, from 45 to 60 years old, with a phototype III (Fitzpatrick grading scale) and loss of skin elasticity on the face.

Evaluation method
The evaluation of biomechanical properties were performed using the Cutometer® SEM 575 (Courage & Khazaka) on both cheekbones with calculation of parameters R2, R5, R7, Q1 and Q3.

R2 (Ua/Uf) and R5 (Ua/Ue) are skin elasticity parameters and R7 (Ur/Ue) is a skin firmness parameter.

Q parameters are surface ratios parameters.

Q0 represents the maximum extension or recovery area, which has the same value in both phases. The area above the recovery curve is defined as total viscoelastic recovery, which consists of an elastic recovery area, QE, and a viscous recovery area, QR. A horizontal line from the inflection point separates these two areas. Dividing area QE by the maximum recovery area Q0, we obtain the elastic recovery dimensionless parameter Q1 = QE/Q0. Dividing these areas QE and QR by the maximum recovery area Q0, we obtain the viscoelastic recovery dimensionless parameter Q3 = (QR+QE)/Q0. Q1 and Q3 parameters decrease with age [12] and are related to a loss of skin firmness.

Results and statistics
The results were expressed as the mean percentage of variation of the parameter: \((D56-D0)/D0\). The mean percentages of variation compared to placebo were calculated as mean variation Argassential™ at 2% - mean variation placebo.

Statistics: the normality of distribution is checked using Shapiro-Wilk test. Statistical comparison of the evolution of the parameter \((Dx-D0)\) is done with Student’s t tests (if the normality of distribution is confirmed) or with Wilcoxon test (if the normality of distribution is rejected). Level of significance is 5%.

Study on the face of the plumping effect – Evaluation of lateral profiles on cheekbones by 3D Primos® Body

Study design
The clinical study was carried-out as a placebo-controlled double-blind randomized study on two groups, one with lipstick containing Argassential™ at 2%, the other with placebo lipstick. The formulations are detailed in Annex 2. The study was conducted during a period of 28 days with check points at D0 and D28. This study was carried out from February to March.

Inclusion criteria
The tests were done on 21-22 female Caucasian volunteers, from 40 to 65 years old, with lips neither too fine nor too voluminous.

Evaluation method
The products were applied by the volunteers twice a day during 28 days, under the normal condition of use. The last application was the evening before each visit.

Results and statistics
The lips volume (mm3) was evaluated by fringe projection using PRIMOS ® 3D PICO at baseline and after 28 days of treatment. Results were expressed as the mean volume of lips at D0 and D28. The volume variations were expressed as the mean percentage of variation \((C28-D0)/D0\). Statistical analysis was carried out in the same manner as above.
General conclusion

Argassential™ is a hydro- and lipo-dispersible extract rich in esterified triterpenes, obtained through supercritical extraction of a by-product of the production of argan oil, the fruit pulp.

This cosmetic active ingredient is a part of the BASF’s Argan sustainability program based on a long term and responsible partnership with women cooperatives in Morocco.

Argassential™ offers a dual action:
- it boosts Collagen type I in vitro, to density the dermis and firm the skin,
- it fights against “adipocytes ageing” by increasing differentiation and lipid storage in vitro, to rejuvenate the adipocytes and plump the skin.
Annex 1 - Technical data available upon request

- Quality and Regulatory Product Information
- Composition sheet
- Specifications
- Formulation Data Sheet
## Firming effect on the face

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Plumping effect on the face
### Plumping effect on the lips

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