Argania spinosa –
How Ecological Farming, Fair Trade and Sustainability Can Drive the Reasearch for New Cosmetic Active Ingredients

Keywords: argan, sustainability, active ingredient
The Argania forest: socio-economic role

The Argania forest has a very important socio-economic role in these regions. Its special legal status (Dahir of March 4th 1925 and the specifications for agricultural practices under Argan trees of July 20th 1983) has made it a state owned forest with extensive rights of use reserved for the local populations: right to harvest fruits and collect wood for personal use, right to free passage. At the same time, the right to cultivate is subject to authorisation by the local Eaux et Forêts Administration (Water and Forest Government Agency) and a fee has to be paid. Furthermore, each village is obliged to keep its trees in good condition. It should not be forgotten that the Moroccan Argan provides subsistence for over 3 million people in a country with a population in excess of 30 million. It is the focus for many rural activities.

The inhabitants of the Argania forest benefit both from being able to use the different parts of the tree (fruits, leaves, wood) for their own needs and also from the work and associated revenue that its exploitation provides.

The fruits, which are mainly used for producing the oil, also provide part of their livestock’s alimentation via the production by-products: the oil cake is given to the goats and the husks are used as fuel for fires. The oil is sold locally and has seen its use and commercialisation become widely developed at an international level.

The development of agriculture under the trees has led to Argan exploitation becoming more profitable, sometimes to its cost. It does however mean that local populations are better nourished and have improved incomes.

It is suggested that exploiting the Argania forest provides 20 million days of work per year, allowing rural populations to stay in the countryside and limiting the rural exodus.

A fragile ecosystem

Unfortunately the Argania forest is fragile, partly as a victim of its riches but also because of changes in the rural way of life and climate. Over-cultivation, soil erosion and advancing desertification are amongst the threats to this unique heritage. The natural increase in the population leads to an increase in wood collection, which is used for both construction and as firewood.

On the plains, the amount of land used for agricultural purposes is growing and this is accompanied by scrub clearance and installation of irrigation systems, which result in underground water reserves running dry and increasingly salty soil. The groundwork, which often goes deep, damages the Argan tree root systems and leaves them weakened. Excessive grazing makes it more difficult for the Argans to recover. The climate, which is always hard in these regions, is accompanied by erosion that makes the soil even more arid.

Implementing actions to preserve and regenerate the Argania forest is therefore a priority, as much from an ecological point of view to preserve this last barrier against the advancing desert as from an economic perspective to maintain a source of revenue for the local population.

UNESCO recognised this issue and classed the Moroccan Argania forest as a Biosphere Reserve (MAB) in 1998. The Biosphere Reserves are delimited regions in land-based ecosystems where one tries to reconcile economic development with conserving the biodiversity. Biosphere Reserves serve in some ways as ‘living laboratories’ for testing out and demonstrating integrated management of land, water and biodiversity, which is the principal of the ecosystem approach that has resulted from the biological diversity convention (UNESCO 1996).

The Biosphere Reserves have to fulfil three main tasks that are complementary and synergistic:

- conservation role: contribute to the conservation of the whole range of biodiversity elements especially the landscapes, the ecosystems, the species and the genetic variation,
- development role: to foster economic and human development, which is socio-culturally and ecologically sustainable,
cosmetic actives through fair trade and sustainability

To Laboratoires Sérobiologiques, sustainability and fair trade are core values, that have driven the Argan program.

The Argan forest is one of the most important natural treasures of Morocco. It acts as a natural defense against the encroaching desert and plays a vital role in maintaining ecological equilibrium and preserving biodiversity in Northern Africa.

It is threatened by extensive exploitation and urbanization. The only ethical and long-term approach to preserve it is to support the local population to utilize it in a sustainable way, and to financially encourage environmentally conscious behavior.

- ARGANYL:
  Polyphenols from the leaves: anti-age properties based on anti-collagenase and anti-MMP activity

- ARGATENSYL:
  Proteins from the fruit: tightening properties

- LIPOFRUCTYL ARGAN:
  Precious Argan oil with ECOCERT label
The global network of Biosphere Reserves resulted from UNESCO’s Man and Biosphere (MAB) programme, which intends to provide the means required to ensure the conservation and sustainable use of the biological diversity and to improve the relationship between individuals and their environment at a global level. They are internationally recognized, nominated by national governments and remain under sovereign jurisdiction of the states where they are located. In 2004, the global network of Biosphere Reserves consisted of 459 Reserves in almost 100 countries.

**Actions to promote the protection of the Argania forest**

The need to protect the Argania forest has not gone un-noticed either by the local authorities or at an international level. Many initiatives have been launched to help preserve and develop the Argania forest and to reverse its regression. With this aim in mind, Professor Zoubida Charrout from Rabat University, who has campaigned for many years to improve the status of Moroccan rural women, created Targanine in 1996. Targanine is a network of cooperatives whose objective is to save the Argania forest: firstly, by educating the local populations in the protection of their heritage and, secondly, by providing the means for them to organise the traditional production of Argan oil and to develop its commercialisation. The cooperatives, which consist of a partnership aimed at identifying new sources of cosmetic active ingredients but with one over-riding condition: the new developments must support and encourage the protection and add value to the Moroccan Argania forest.

**For fair trade**

The cooperatives agree to sell the fruit of their enterprises at market prices that ensure an adequate return to sustain their activity. Furthermore, from an economic point of view, they ensure that women receive a salary equivalent to the local minimum wage, which is a big step forward compared to the normal situation where traditional production usually uses underpaid labour. Being assured of a decent salary is a measure of social recognition for these women and the cooperatives are also a way of escaping from the isolation that is their usual fare.

**For sustainable development**

The major objective of these cooperatives is to increase awareness amongst local populations of the problems related to the fragility of the Argania forest, which is often their only source of income. In order to develop their business, it is essential that means are found to preserve and regenerate this valuable source.

It is important to support these actions today to ensure the long-term protection of the Argania forest. Using known or new fractions of the Argan for cosmetic purposes directly linked to well being can not be considered unless the complete respect of this weakened ecosystem can be guaranteed.

For the sustainable development of this business, the collection conditions must be rational and strictly monitored. For this reason, Laboratoires Sérobiologiques have launched a number of initiatives in this respect in collaboration with recognised local actors.

**A partnership based on fundamental values**

For many years Laboratoires Sérobiologiques have applied ethno-botanic and sustainable development methods. After their experiences in French Guyana (Flux-hydran® programme), they have become involved in initiatives for the preservation of the Moroccan Argania forest. For nearly 4 years, Laboratoires Sérobiologiques and the Targanine network of cooperatives, under the initiative and with the close collaboration of Professor Zoubida Charrout, have been developing a partnership aimed at identifying new sources of cosmetic active ingredients but with one over-riding condition: the new developments must support and encourage the protection and add value to the Moroccan Argania forest.

This North–South partnership is centred on three main elements:

- Identification of specific fractions (leaves and oil cake) of the Argan that could be of value to the cosmetic industry and which will provide additional incomes for Moroccan women.

- Perform a study to evaluate the effects of harvesting leaves on Argan growth and to define the most appropriate specifications.

The environmental effect of adding value to the leaves depends on the tree’s ecology, the dynamics of the ecosystems and the socio-economic circumstances. The study looked at these different factors during a three-month mission to Morocco in 2004, which Laboratoires Sérobiologiques financed.

The response of the Argan to its leaves being harvested for their potential cosmetic value was studied by installing an experimental unit in the village of Amelne, Tiznit province, in the South of Agadir. This work was done after having received approval from the provincial governor and the local authorities. The experimental unit, established on a plot of the Argania forest, involved monitoring growth, flowering and fruit production of trees, which were subject to a range of sampling protocols. As the results were not expected until after sev-
eral years of study, other ways of obtaining Argan leaves were also looked at, including short-term measures for Argania forest preservation that could be used by the Targanine cooperatives. There are several options for collecting leaves:

- Collection of yellowing leaves on the ground or on the trees,
- Picking green leaves on the trees,
- Recovering leaves from maintenance operations on the Argania forest: coppicing.

After evaluating several possibilities, the practice of coppicing was selected. This maintenance operation has to be done systematically and regularly and involves selecting the best stems on each tree and then removing the others. This leads to a spontaneous regeneration of the Argan and the branches that are kept develop with greater vigour and are highly productive. This provides a source of leaves that can be collected and dried. The local population uses the wood that is produced. This procedure, which has been approved by the local authorities, has a positive effect on the environment because it provides a perpetual improvement to the local population but will also ensure the long-term sustainability of the existing Argania forest. Other types of plant (ornamental, and fodder herbs) are also part of the program and will provide the local populations with complementary incomes.

The result of a North–South collaboration: the »LS Argan Program«, a range of cosmetic active substances from the Argan tree for skin care. Whilst the Argan is mainly known for its oil, Laboratoires Sérobiologiques have extended their field of work to look at other fractions. By combining local traditional knowledge with their scientific expertise, they have developed a complete range of several active ingredients for cosmetics for skin care that have been created whilst respecting the fundamental values involved in sustainable development and fair trade.

### Argan leaf extracts: the polyphenols

So as to encourage the local population to mark out the Argania forest as required by the impact study, the potential value of the leaves using phyto-chemical analysis was evaluated. The Argan leaves have a high concentration of polyphenols, known for their anti-free radical properties and for the prevention of skin ageing. HPLC PDA identification of the following components:

- Myricitrin (main component)
- Rutin
- Quercitin
- Quercetine-3-O-galactoside

A series of tests has demonstrated their anti-collagenase, anti-MMP-1 and anti-free radical properties. A consumer test has shown its potential for protecting the skin against premature ageing.

### MMP and skin ageing

The skin’s structure, especially its firmness and elasticity, is directly related to the composition and structure of the ECM (extra-cellular matrix). This extra-cellular compartment consists mainly of fibrous macromolecules: collagen and elastin that are synthesised by fibroblasts. It is a place of intense biological activity and exchange. Many enzymes are active in this compartment in a sometimes-fragile equilibrium that is optimal in the young skin. We are talking about homeostasis.

The MMP (Matrix Metallo-Proteinases) are enzymes synthesised by fibroblasts. Their role is to cause the slow degradation of collagen fibres so as they can be renewed. Tissue inhibitors called TIMP counterbalance their action. They work in a combined and balanced fashion.

During skin ageing, there is an imbalance between MMP and TIMP with an increase in production and activation of MMP and a reduction in TIMP, which is a breakdown in the homeostasis system. This leads to an increased rate of degradation of collagen fibres, and a loss of density in the ECM. At the same time, fibroblast collagen synthesis slows down. The direct result is a loss of skin elasticity and firmness. This phenomenon is aggravated by exposure to the sun: many bibliographic studies have shown that MMP are over expressed in sun-exposed skin which aggravates actinic ageing (Fig. 1). Arganyl™ is able to work against the damaging effect of MMP on the collagen fibre network and thereby preserve the quality of the tissues supporting the skin. This action is complemented by an upstream effect due to its anti-free radical properties. These free radicals, whose production is boosted by environmental stress such as UV exposure, also contribute to the skin ageing process (Fig. 1). It is a new and natural solution for protecting skin against premature ageing and especially UV induced ageing.
Demonstration of anti-MMP-1 properties

MMP-1 (Matrix-Metallo-Proteinase type 1) is a collagenase secreted by dermal fibroblasts. Its concentration is increased in aged skin. It is also greatly increased by UV exposure or during inflammation. It initiates a degradation of fibrous collagen (types I and III) in fragments which are further cleaved into small peptides by MMP-2 and MMP-9. Moreover, MMP-1 expression is induced by ROS (Reactive Oxygen Species), UVA and B, therefore MMP-1 play a major function in cutaneous ageing and photo-ageing.

The aim of this trial is to evaluate the potential inhibiting effect of Arganyl™ on this class of MMP.

Protocol

- Reactive mixture at pH 7.6: Human MMP-1 + Arganyl™
- 60 minutes of incubation at 20°C
- Addition of synthetic MMP-1 substrate: Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH2
- Fluorescence measurement at 393 nm
- Estimation of enzyme activity inhibition.
- A positive control is used to validate the test (TIMP-1)

Results (Fig. 2)

Arganyl™ has demonstrated a direct dose dependent anti-MMP-1 effect that confirms its ageing prevention properties.

Demonstration of anti-collagenase properties in-vitro

The aim of this trial is to confirm the anti-collagenase properties observed in laboratory tests (IC 50 = 0.55%). In this trial, human skin biopsies are incubated with collagenase: the collagen network, made visible by fluorescent antibodies, shows a massive loss of collagen fibers. The ability of Arganyl™ to prevent this destruction was compared to phenanthroline, a positive anti-collagen control (Fig. 3).

When Arganyl™ is added at a dose of 0.5%, the dermis retains its initial appearance with a dense and uniform collagen network. Furthermore, this protective effect against collagenase is dose dependent.

Demonstration of anti-free radical properties

In order to complete our understanding of the anti-ageing effects, its ability to protect the skin against oxidative ageing of its anti-free radical properties have been demonstrated.

Free radicals play a predominant role in skin ageing, as much at the cellular level as in the ECM. They are particularly important in the oxidation of the macromolecules that make up the ECM, leading to a degradation of the skin’s physical properties. Additionally, the free radicals, especially the ROS generated by UV exposure, can stimulate MMP expression by dermal fibroblasts.

The anti-free radical ability has been evaluated using a variety of laboratory and in-vitro tests. The tests looked at both the natural initial forms and the induced reactive forms of oxygen. Arganyl™ has demonstrated a significant anti-free radical activity especially against the oxygen reactive forms such as hydroxyl radicals (HO°) and superoxide anions (O2•-) (Fig. 4).

Consumer tests

The laboratory and in vitro tests described above were completed by consumer tests. Fifteen 45 – 65 year’s old women were involved in this study. Each applied the two creams described below to their faces twice a day, morning and evening, for 8 weeks:

- placebo cream on one side,
- 3% of Arganyl™ cream on the other side.
At the end of 8 weeks, they were asked to evaluate the benefits of the treatment comparing before and after treatment and treatment against placebo. 88% of the women thought their skin had more elasticity. 71% of the women thought the grain was finer and the pores less obvious. 100% of the women thought their skin was softer to touch. These different factors were quantified on an arbitrary scale from 0 to 3 in comparison with the placebo. The results are summarized in Fig. 5.

**Conclusion**

Arganyl™ extracted from Argan leaves is rich in polyphenols and has proven its protective effect on the ECM via an identified biological mechanism acting on collagenase such as on MMP-1 to provide overall collagen protection. This biological effect results in the prevention of skin slackening that can be detected by consumers. It is an active ingredient that is especially suitable for anti-ageing care of the face but can also be used to return firmness to the body. It also has an undeniable potential in anti-age sun care products.

- **Fruit extracts: 'Almond' Kernel – The oil**

Argan fruits have a very hard shell that encloses one to three almond like kernels from which the precious oil is extracted. Only women are involved in preparing this oil. On average, 33 kg of dry fruit, harvested from 6 to 7 trees, are needed to make 1 litre of oil. Lipofructyl Argan™ is prepared by mechanical extraction (cold pressing) and satisfies the criteria of 'extra-virgin'. Thanks to the special efforts of the Tar-ganine network, this oil has been certified ECOCERT and received the Slowfood 1st prize in 2001.

In terms of its composition, Argan oil is ex-
exceptionally rich in poly-unsaturated fatty acids (including linoleic acid, omega-6) and natural tocopherols.

Average composition:
- Fatty acids: 99%, of which over 80% is unsaturated fatty acids
- Insaponifiables: 1%
- Tocopherols: 60 - 90 mg/100 g
- Sterols: 130 - 230 mg/100 g

Fatty acid composition
Thanks to its exceptional concentration of unsaturated fatty acids, Argan oil helps restore the skin’s hydro-lipid film. Linoleic acid, one of the omega-6 fatty acids, is a vitamin F precursor. It is present at a concentration that is three times greater than in olive oil. As an essential fatty acid, it has an important role in preserving cellular integrity. The concentration of natural tocopherols is especially high and gives the oil excellent anti-radical and anti-stress properties in addition to natural self-protection against oxidants (Fig. 6).

Argan oil is widely used by Moroccan women, both in their cooking for its taste and for many traditional beauty rituals, for skin, hair and nail care, and has been for centuries. Many traditional beauty secrets are based on Argan oil; e.g. Berber women cover themselves in a mixture of equal parts of Argan oil and almond oil before going to the hammam (steam baths).

Skin care
When applied to the skin, Argan oil rapidly penetrates without leaving a greasy or shiny trace. In fact, its effect is completely the opposite as it leaves the skin soft with an extraordinary sensory feeling. As a body care product, the oil is ideal for massages. It provides:
- an excellent anti-free radical effect that provides preventive anti-ageing care,
- a nutritional effect on dry skin with a tendency to eczema by helping to restore the cutaneous barrier,
- a sebum regulating action for greasy skins,
- a regenerating ability for the care of stretch marks and enhancing healing.

Fig. 6 Fatty acid composition of argan oil

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Average Composition</th>
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<tbody>
<tr>
<td>Palmitic acid</td>
<td>(C16:0): 12 - 13%</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>(C18:0): 5 - 7%</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>(C18:1): 43 - 49.1%</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>(C18:2): 23.9 - 36.0%</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>(C18:3): &lt; 0.1%</td>
</tr>
<tr>
<td>Arachidic acid</td>
<td>(C20:0): 0.3 - 0.5%</td>
</tr>
<tr>
<td>Gadoleic acid</td>
<td>(C20:1): 0.4 - 0.5%</td>
</tr>
</tbody>
</table>

Technical expertise, state-of-the-art equipment, stringent certified quality management, and exceptional commitment – these are our strengths in clinical trials.

When you choose proDERM, your dermatological study is in reliable hands: you’ll find competent staff at your disposal, who can provide you with expert advice and assistance; we look forward to working with you!
Hair care
Argan oil is traditionally used as a mask or hair conditioner as it:

- strengthens hair, provides shine, softness and a silky effect,
- nourishes and revitalises the scalp.

Nail care
Argan oil is commonly used in a mixture with equal parts of lemon juice as an evening application for nails before going to bed. Regular use of the mixture strengthens brittle nails.

Argan oil, considered as 'Berber’s gold', is a precious oil with multiple and varied cosmetic uses.

The proteins
So as to increase the profitability of the cooperatives producing Argan oil, the cosmetic use of the by-products of this business was evaluated.

A fraction rich in high molecular weight proteins (> 200 000 Daltons) has been identified and isolated from the oil cake. Its composition gives it very interesting surface properties. When it is applied to the skin, the molecules form a network with a high affinity for the cutaneous surface and develop an instant and perceptible tightening effect.

Argatensyl™ is a cosmetic active ingredient constituted exclusively from this specific active fraction, which is present in a water-soluble form that makes easier its incorporation into finished products. Its instant skin tightening properties and its ability to correct wrinkles, have been demonstrated by the clinical trials described below.

Consumer tests
Immediate tightening effect – Crow’s feet area

Application of an oil-free serum, containing 5% or 10% Argatensyl™, on the crow’s feet in a group of fifteen 35 – 45 year’s old women.

Macrophotographs taken at T = 0, T + 15 minutes and T + 1 hour. The trialists used a semi-quantitative scale to make a visual evaluation of the tightening effect. The results are shown in Fig. 7 and Fig. 8.

Fig. 7 Before and after application of a serum containing 5% Argatensyl™

Fig. 8 Semi-quantitative evaluation of the benefits of Argantensyl™ treatment compared to a placebo

Fig. 9 Before and after application of a serum containing 5% Argatensyl™, on neck wrinkle
Long term anti-wrinkle properties – neck wrinkles
Application of an oil-free serum, containing 5% Argatensyl™ to the neck, in the morning and the evening for 4 weeks by a group of fourteen 35 – 45 year’s old women.
Macrophotographs and prints before treatment and after 28 days of treatment were taken to evaluate depth of wrinkles.
The results are shown in Fig. 9 and Fig. 10.
Conclusion
Argatensyl™ has a good anti-wrinkle effect when applied for a long term treatment of 28 days. Wrinkles are significantly reduced.
The Argan proteins have a special interest for products with instant tightening properties for a ‘flash’ effect especially on crow’s feet wrinkles as well as for anti-ageing and anti-wrinkle facial care products. They can also be used in products for the neck, the décolleté and for the body.

Conclusion
The Argan, the tree that symbolises Morocco, is an incontestable source of richness however we must understand how to preserve it so as to ensure its long term existence and preserve the ecological and economical future of this part of Morocco.
The »LS Argan program« combines identifying sources of new and attractive active ingredients for the well being of everyone whilst respecting man and his environment, using concrete actions that favour sustainable development and, above all, the conservation of the Argania grove.

References
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Fig. 10 Improvement of skin surface, and decrease of neck wrinkle after 28 days of treatment