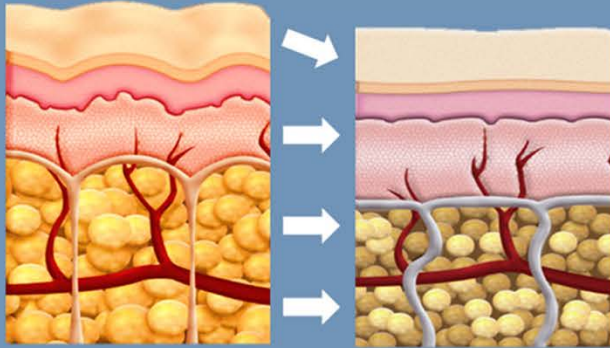


adipo-trap

a carnivorous plant to reshape the body



in vitro

- reduces fat accumulation during adipogenesis
- reduces adipocyte size with a lipolysis effect

within-patient clinical trial

- slimming and reshaping effect –1.4 cm thigh circumference in 2 weeks
- starts acting on cellulite in just 2 weeks
- reduces cellulite-related pain in just 2 weeks

Abstract

ADIPO-TRAP is a slimming, re-shaping and anti-cellulite active ingredient derived from sundew, a carnivorous plant characterized by a phytocomplex with two main molecular peculiarities: the adhesive polysaccharide secretion of the trap and secondary metabolites with anti-inflammatory and bio-active properties.

During *in vitro* tests performed using preadipocyte and adipocyte cell models, ADIPO-TRAP was able to reduce the fat accumulation during the adipogenesis process and to reduce the adipocyte size, showing an efficient lipolysis effect.

During a within-patient clinical trial performed on 20 volunteers, ADIPO-TRAP was shown to be able to act as a slimming and reshaping active ingredient, reducing thigh circumference by 1.4 cm in just 2 weeks.

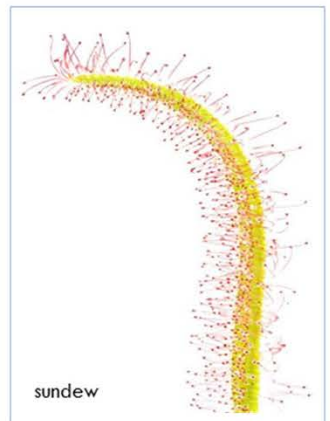
ADIPO-TRAP was also able to start reducing cellulite and cellulite-related pain in just 2 weeks.

Carnivorous plants: a wonder of nature

Carnivorous plants have always fascinated both botanists and common people, and scientists were reluctant to accept that plants, historically considered "inferior" in the natural food chain, could be able to prey on animals. Charles Darwin (1809-1882), mostly known for his revolutionary *On the Origin of Species* (1859) regarding the theory of evolution, was also an esteemed botanist and was the first scientist to provide detailed experimental evidence of carnivory in several plants.

Plant carnivory is nevertheless a rarity and occurs in only about 0.2% of plant species (550-600 out of approximately 250,000). In general, these plants grow in sunny areas with mineral-deficient soil, quite often with gently moving water. These habitats are generally low in nitrogen and phosphorus and therefore carnivory is an alternative strategy to obtain essential nutrients.

Carnivorous plants are characterized by two main features: the ability to trap prey and the adaptation of their metabolism to their unusual diet/habitat. As often happens, when a plant grows in extreme habitats or stressful conditions, its metabolism is forced to produce some uncommon secondary metabolites. These are the usual conditions for carnivorous plants that have thus developed the energy-expensive carnivory strategy in order to occupy extreme ecological niches. The nutrient-poor habitat, the specific chemical and morphological adaptations related to the trapping process, and the different source of the nutrients derived from prey has led to the development of very peculiar phytocomplexes. Traps of carnivorous plants are true marvels, these generally represent a modification of leaves and can be classified as active or passive.



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Sundews: active plants with passive traps

Drosera is one of the largest genera of carnivorous plants comprising more than 190 species. Under the common name of sundews, a large variety of plants which vary greatly in size and form, can be found growing natively on every continent except Antarctica. These plants generally grow in seasonally moist or more rarely constantly wet habitats with acidic soils and high levels of sunlight. Sundews are perennial herbaceous plants that have been shown to be able to achieve a lifespan of 50 years.

Sundews are all characterized by the glandular tentacles, topped with sticky secretions, that cover their leaves. All species of sundew are able to move their tentacles in response to contact with prey. The tentacles are extremely sensitive and will bend toward the center of the leaf to bring the victim into contact with as many stalked glands as possible.

One of the most distinguishing features of sundews is therefore the ability to produce an adhesive secretion on the tip of the tentacles. This mucilage is made up of high molecular weight acidic polysaccharides that give the gel its sticky property. These polysaccharides consist mainly of D-glucuronic acid, which gives the gel its acidic properties, and D-mannose, with lower concentrations of D-galactose, L-arabinose and D-xylose. Some naturally occurring polysaccharides have recently been evaluated as novel interesting biomaterials for tissue engineering. Some research groups have focused their research on the ability of the adhesive polysaccharides naturally secreted in sundew traps to support cell growth. Researchers have demonstrated the ability of these bio-materials to support soft and hard tissue repair during some *in-vitro* tests, confirming the ability of sundew trap secretion to act as a promising support for the purpose of tissue engineering. Carnivorous plants such as sundews have been used through the centuries in traditional medicine for the specific phytochemicals that these plants have developed due to their peculiar characteristics.

Therapeutic properties of sundews are attributed to compounds of the group of acid phenolics, naphthoquinones and flavonoids mainly accumulated in the plant tissues constituting the trap. Some researchers have demonstrated that flavonoids like quercetin, hyperoside and isoquercetin extracted from sundew were able to inhibit a human neutrophil elastase, a proteolytic enzyme acting on collagen IV and elastin in the extra cellular matrix involved in some degenerative and inflammatory diseases. Sundew-derived products are therefore also very interesting for several cosmetic applications due to their anti-inflammatory effects.

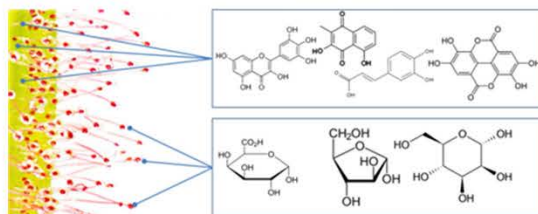


ADIPO-TRAP: a carnivorous plant to reshape the body

Sundew is characterized by two main molecular peculiarities: the adhesive secretion of the trap and the secondary metabolites with anti-inflammatory and bio-active properties. These two fractions were specifically studied to develop a bespoke enzymatic treatment to achieve the complete recovery of the whole phytochemical in a biologically active form with a bioliquefaction process. The mucilage trap is made up of high molecular weight acidic polysaccharides. These molecules have an high molecular weight, one of the critical factors in producing the sticky properties, is also the main obstacle for skin penetration.

Some strictly controlled hydrolytic treatments were performed in order to achieve some specific molecular sizes for these kind of polysaccharides in order to allow them to reach the lower layers of the skin and act as supporting bio material to improve the cellulite-affected skin conditions.

A number of specific enzymatic treatments were also used in order to release all the secondary metabolites since some of these, such as the acid phenolics that might be associated with the solid structures of cell walls and the flavonoids that can be in glycosylated forms, can be either difficult to extract or insufficiently biologically active.

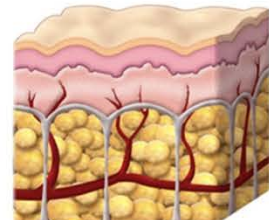
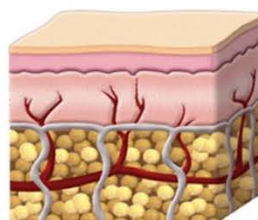


Adipocytes, adipogenesis and cellulite

Adipose tissue is loose connective tissue composed mostly of adipocytes. In addition to adipocytes, adipose tissue contains the stromal vascular fraction of cells including preadipocytes, fibroblasts, vascular endothelial cells and a variety of immune cells. Its main role is to store energy in the form of lipids, although it also cushions and insulates the body. Far from hormonally inert, adipose tissue has, in recent years, been recognized as a major endocrine organ, as it produces hormones such as leptin, estrogen, and the cytokine TNF α . Moreover, adipose tissue can affect other organ systems of the body and may lead to disease.

Adipose tissue is derived from preadipocytes. Adipogenesis is a tightly regulated cellular differentiation process by which an undifferentiated preadipocyte is converted to a fully differentiated adipocyte. Lipid accumulation increases throughout the lipidogenic process and it is regulated by genetic and growth factors.

Cellulite (also known as adiposis edematosa, orange peel syndrome, etc.) is the herniation of subcutaneous fat within fibrous connective tissue that manifests topographically as skin dimpling and nodularity, often on the pelvic region (specifically the buttocks), lower limbs, and abdomen. Cellulite occurs in most post-pubescent females and is thought to occur in 80-90% of post-adolescent females is therefore a relevant matter of cosmetic interest for a large number of individuals. In fact, the irregularity of the natural relief of the skin is an undesirable condition and aesthetically unacceptable for most post-adolescent women. Cellulite can affect any area of the body that contains subcutaneous adipose tissue. Some areas are, however, more sensitive, such as the back and outer thighs and buttocks, but it can also be found on the lower belly, upper arms and neck. As the adipocyte cells grow in size, the surrounding tissue becomes compressed and hardened, making the circulation of fluids more difficult.



representations of a healthy skin with normal adipose tissue (left) and of a cellulite-affected skin with larger adipocytes due to an excess of fat accumulation in the adipocytes.

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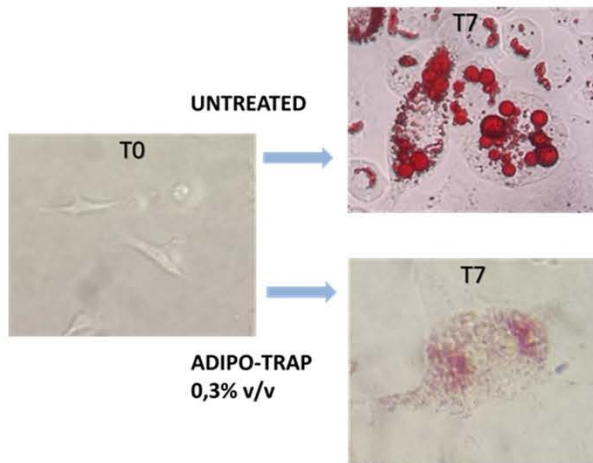
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ADIPO-TRAP: *in vitro* tests

In order to assess the capacity of ADIPO-TRAP to intervene in lipid metabolism and the adipocyte cell cycle, some specific *in vitro* assessments were performed. The cell line model used was 3T3-L1. These cell line is one of the most common and reliable models for the study of the conversion of preadipocytes into adipocytes. The adipocytes obtained at the end of the differentiation process are also used to assess the performances of adipocytes treated under different conditions.

Adipogenesis studies

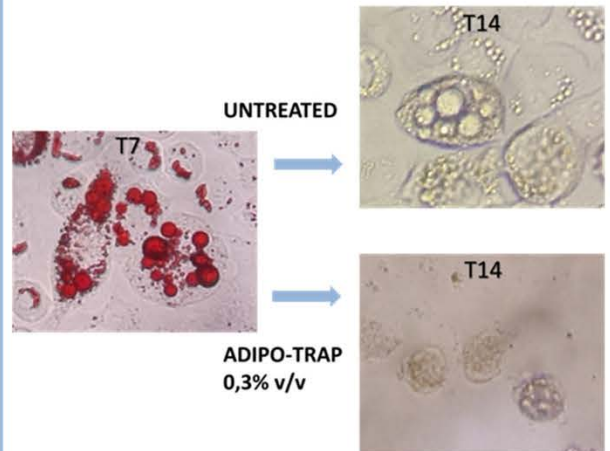
The first target of our assessment was adipogenesis that involves the process of differentiation to adipocytes starting from pre-adipocytes. For these tests, the 3T3-L1 cell lines were grown according to a standard differentiation protocol¹⁵ adding 0.3 % v/v of ADIPO-TRAP in the treated sample to compare to the untreated one as a typical differentiation process. ADIPO-TRAP was absolutely non-cytotoxic at the tested concentrations for preadipocytes during adipogenesis, so any effect highlighted by this test is due to metabolic path modulation and not to cytotoxicity. As shown in Figure 6, at day 7 after completing the differentiation protocol, the untreated adipocytes are fully differentiated and are already showing some characteristic lipid droplets. The cells treated with ADIPO-TRAP are also completely differentiated into mature adipocytes, but the content of triglycerides is far lower than that of the untreated cells. Even the staining process with the Oil red O, used to make fat more visible, does not make it possible to identify the presence of large droplets of triglycerides but just the content of widespread very small fat droplets. ADIPO-TRAP is therefore already able to reduce fat accumulation during adipogenesis, the differentiation process from pre-adipocyte to adipocyte.



images of pre adipocytes (T0) and adipocytes (T7) after the differentiation process (adipogenesis). The Oil red O staining shows a clear decrease of the lipid content in differentiated adipocytes in the presence of ADIPO-TRAP 0.3% v/v.

Lipolysis studies

The second target of our experiment was the ability of ADIPO-TRAP to intervene on the amount of fat that is stored in adipocytes. For this set of tests fully differentiated adipocytes were obtained with the standard adipogenesis protocol. Differentiated adipocytes collected at day 7 (T7) were used in a lipolysis study. Adipocytes were incubated in a standard growth medium with and without ADIPO-TRAP 0.3% v/v. In these tests too, ADIPO-TRAP was absolutely non-cytotoxic for adipocytes, so any effect highlighted by this test was due to metabolic path modulation and not to cytotoxicity. As shown in Figure 7, after 7 days of incubation with a standard medium the untreated adipocytes were accumulating triglycerides in large droplets that were occupying all the available volume of the cell. Adipocytes treated with ADIPO-TRAP 0.3% v/v were much smaller in size than the untreated ones, showing an efficient lipolysis effect caused by the product that was efficiently reducing the fat content in the cell.



images of adipocytes (T7) and after incubation for 7 days (T14) with a standard growth medium with and without ADIPO-TRAP 0.3% v/v

In our *in vitro* test, ADIPO-TRAP was therefore able to reduce the fat accumulation in adipocytes during the adipogenesis process and reduced the adipocyte size, showing a lipolysis effect.

ADIPO-TRAP: within-patient clinical trial

To evaluate the performances of ADIPO-TRAP as a slimming, re-shaping and anti-cellulite active ingredient for cosmetic applications, a series of clinical trials were performed.

A simple gel formulation containing a bio-liquefied sundew product (3 % p/p) and caffeine (1 % p/p) was compared with the vehicle, the same formulation containing just caffeine (1 % p/p), as control.

Twenty female patients with cellulite of the lower abdomen and the lower limbs of grade II-III were selected.

This was a single-blind within-patient study where 20 volunteers applied the product containing ADIPO-TRAP on the right leg, and at the same time, the vehicle gel on the left leg. After delivery of the 2 products, the volunteers recruited for the study were told to apply them twice a day, morning and evening, like the usual treatments for cellulite thighs, for a total of 30 days.

Demographic characteristics of the patients

Patient characteristics	
	n = 20
Male	0 (0%)
Female	20 (100%)
Age (range)	21 - 50
Age (mean and SD)	31,46 ± 10,6
Type 1 Cellulite	0 (0%)
Type 2 Cellulite	9 (60%)
Type 3 Cellulite	6 (30%)

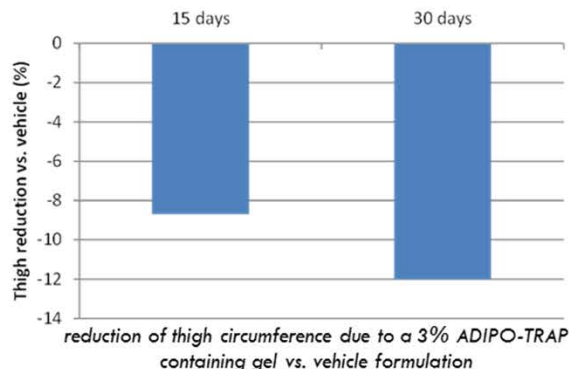
adipo-trap

a revolutionary concept in skin radiance

Anthropometric Measurements

The measurement of thigh circumference is the traditional measure in treatments aimed at reduction of subcutaneous fat. The measurements were made using a flexible and inextensible calibrated tape in mm. Thigh circumference was evaluated in the upper third of the thigh. To facilitate and standardize anthropometric measurements, a landmark was identified on each patient, marked at T0, and at each measurement time if no longer visible, with a marker pen. This point corresponds to the upper third of a line drawn with a metal rule, between the upper edge of the patella and the anterior superior iliac spine. The anthropometric results obtained during the within-patient trial are reported in Figure. The data showed a significant thigh reduction of the leg treated with ADIPO-TRAP compared to the one treated with the vehicle. After just 2 weeks of treatment, there was a clear circumference reduction of - 8.7% compared to the vehicle, after 4 weeks the reduction rose to - 12.0 %.

ADIPO-TRAP reduced thigh circumference by -1.4 cm in just 2 weeks and by -1.6 cm in 4 weeks and is therefore able to quickly act in decreasing subcutaneous fat, thus having a slimming and re-shaping action.



Clinical Dermatology Examination

The subjects' conditions at time zero and during the within-patient study were assessed by skilled dermatologists in order to evaluate the main signs of cellulite. The parameters assessed were: compactness, firmness, pain and orange peel skin. These parameters were evaluated according to a 5 degree scale (0 = absent, 1 = mild, 2 = medium, 3 = notable, 4 = high).

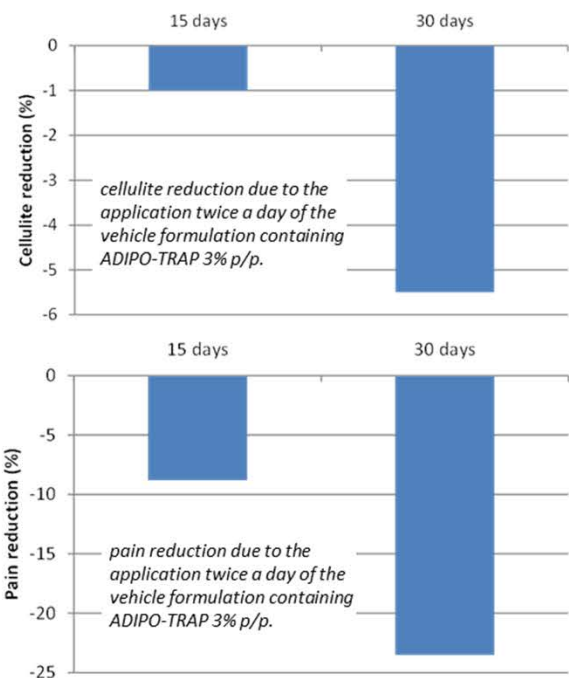
The overall evaluation of these parameters provides information on the cellulite degree of each volunteer and therefore on any anti-cellulite effect of the tested ingredient.

The clinical trial results on the anti-cellulitis effect of ADIPO-TRAP are reported in Figure. The vehicle formulation containing ADIPO-TRAP 3% p/p already showed some effect in 2 weeks, with a -1% reduction of cellulite. After 4 weeks of treatment, the results of the clinical evaluation showed a -5.5% reduction of cellulite.

The vehicle formulation did not show any effect after 2 weeks, while ADIPO-TRAP was already active. After 4 weeks of application, ADIPO-TRAP was 21.3 % more active than the vehicle formulation containing caffeine 1%.

One of the parameters in cellulite that is less advertised in cosmetic products, but that is often perceived by consumers as one of the most annoying, is pain. Cellulite, especially in the more severe forms, is often characterized by a chronic pain sensation. This is also the least subjective parameter to assess in a clinical evaluation, especially in a within-patient trial as the pain threshold is the same for both the treated area and the control as we are dealing with two legs of the same subject.

Results reported in Figure show that the product containing ADIPO-TRAP 3% is able to effectively reduce pain by -8.8 % in just 2 weeks. After 4 weeks of treatment, the product containing ADIPO-TRAP 3% was able to reduce the pain perceived by the volunteers by -23.5%. The vehicle formulation did not show any effect in pain reduction after 2 weeks, while ADIPO-TRAP was already active. After 4 weeks of application, ADIPO-TRAP was 15.2% more active than the vehicle formulation containing just caffeine 1% in reducing pain.



ADIPO-TRAP is therefore a very interesting active ingredient for cosmetics, able to act very quickly as a slimming and reshaping agent. ADIPO-TRAP is also able to promptly reduce cellulite and pain related to it in just 2 weeks.

Technical specifications:

CTFA name (requested): Hydrolyzed Drosera Ramentacea Leaf

Composition: glycerin, Hydrolyzed Drosera Ramentacea Leaf, citric acid, sodium benzoate, potassium sorbate.

Suggested concentration of use: 3-5 % w/w



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