

# An Extract from a Hairy Root Culture from Basil to Treat Hair Loss

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## Summary

Androgenic alopecia is caused by an increased sensitivity of the hair follicles to the androgenic hormone dihydrotestosterone (DHT). It occurs in men and women, is hereditary, permanent and continues with age. An extract of hairy roots from *Ocimum basilicum* (basil) was tested on its activity to inhibit 5 $\alpha$  reductase, the key target enzyme in the inhibition of hair loss. The extract was found to stimulate the proliferating cells in the hair bulb and thus to reactivate hair growth and at the same time inhibiting the hair loss enzyme 5 $\alpha$  reductase II. In a clinical study, the extract of hairy roots from *Ocimum basilicum* showed the capacity to reduce hair loss by 26 % after the first month and 31 % following the second month. Results suggest that the hairy roots basil extract significantly reduces hair loss and thus leads to denser hair.

## Introduction

As consumers continue to strive on healthy and good looks as they age, they are looking beyond skin care. Hair aging is thus playing an increasingly important role. Aging of hair comprises different aspects: Thinning of hair, hair loss, dullness, color fading and graying are all part of this process. Whereas grey hair has gained improved acceptance, thinning hair will always be perceived as negative and therefore hair care products with anti-hair loss claims are of growing interest for everyone.

Hair loss can occur for a variety of reasons such as infections, thyroid and hormonal imbalances, nutritional deficiencies, stress, trauma, drugs, or it may occur as the result of an autoimmune phenomena. Many of these hair loss problems are temporary, although there is another type of hair loss that is less dramatic and less visible but can be incredibly distressing. This involves the hair thinning gradually, often over the course of several decades. It can start at any age, it is progressive and hereditary. Androgenic alopecia, as the condition is known, is male hormone-related but it is not primarily caused by excessive testosterone. Instead, the hair follicles become more sensitive to the hormone due to a genetic predisposition. An enzyme, 5 $\alpha$  reductase, leads to the conversion of the male sex hormone testosterone into the more potent androgen dihydrotestosterone (DHT). This hormone causes the hair follicles to produce thinner and smaller hair, until the point that the hairs stop growing (Fig. 1).

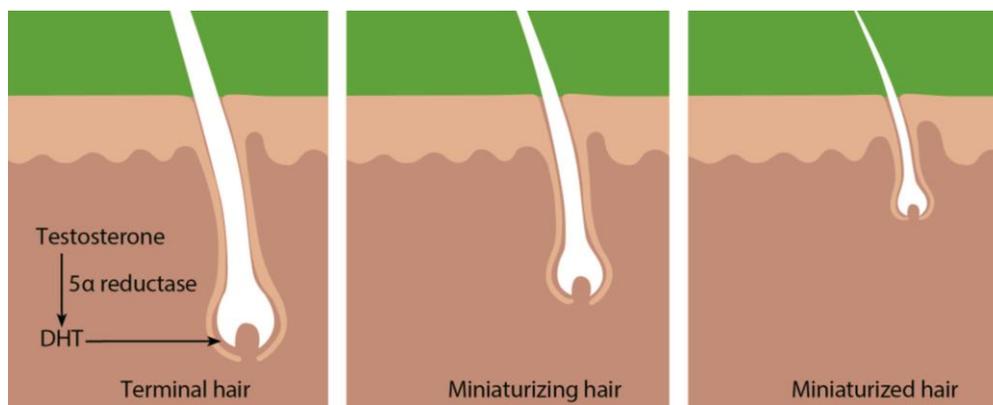


Figure 1: Miniaturization of scalp hair due to androgenic alopecia.

Two isoforms of 5 $\alpha$  reductase are known with a different distribution in tissues and developmental stages: 5 $\alpha$  reductase type I is the principal isoenzyme in sebaceous and sweat glands. 5 $\alpha$  reductase type II has been detected in different parts of scalp hair follicles (1)(2). Balding scalps contain increased 5 $\alpha$  reductase type II activity and dihydrotestosterone (DHT) levels. Men are considerably more affected by hair loss (statistics suggest up to 70 % of men experience hair loss). But it is not only men but also women who can suffer from androgenic alopecia. Meanwhile, approximately 30 – 40 % of women experience hair loss, although androgens are typically present in much smaller amounts. In contrast to men, hair loss in women occurs less in the area of the receding hairline or tonsure, but instead appears on the top side of the head and through the spotty formation of gaps or diffuse hair loss.

Male pattern baldness can be treated with the synthetic drug Finasteride which was licensed 1998 (3). However, diverse side effects are known and the use of Finasteride for female pattern hair loss is controversial. The objective of our research was to find a natural cosmetic ingredient with similar activities as Finasteride that can be used to prevent or counteract hair loss in men and women.

In our studies we tested an extract of hairy roots from *Ocimum Basilicum* (basil hairy roots extract) on its activity to inhibit 5 $\alpha$  reductase, the key target enzyme in the inhibition of hair loss.

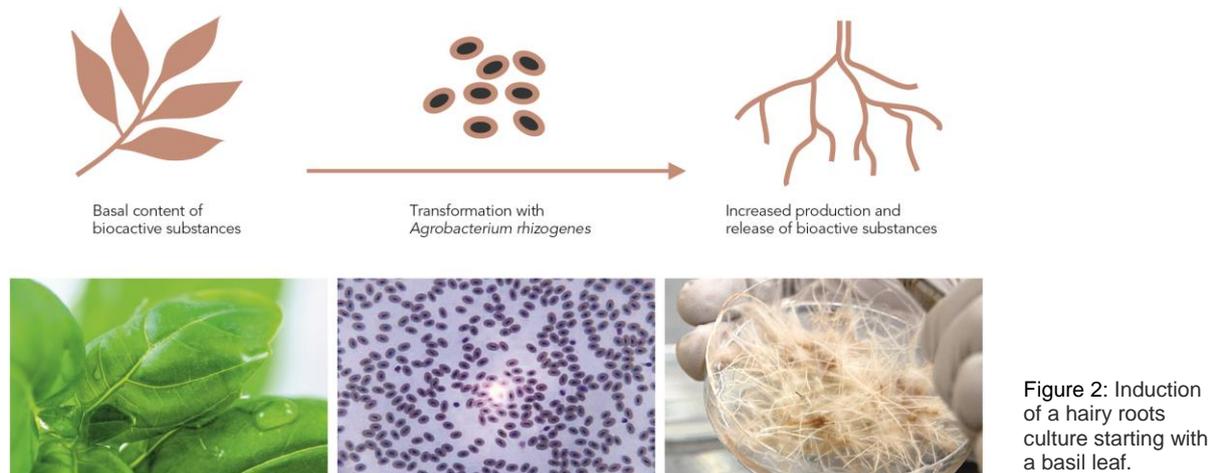


Figure 2: Induction of a hairy roots culture starting with a basil leaf.

## Methods

To produce basil hairy roots extract a basil leaf was infected with the soil bacterium *Agrobacterium rhizogenes* and cultivated on an agar plate. The transformed leaf cells then started to grow tiny roots – the so-called “hairy roots” (Fig. 2). The hairy root cultures were further propagated in specifically designed bioreactors. Roots were then harvested and extracted in an appropriate medium according to a standard protocol.

To prepare 5 $\alpha$  reductase type I and II containing cell fractions, transfected human embryonic kidney cells (HEK I and II) were used. Protein content was quantified using the commercially available RotiQuant® reagent. The fractionated cell suspension was stored at – 80°C. For the 5 $\alpha$  reductase assay, incubations were performed at 37°C in a Tris HCl-EDTA-buffer containing NADPH, androstenedione, cell homogenate and extract from a Hairy Root Culture from Basil. Finasteride, used as a positive control, was dissolved in ethanol and further diluted in Tris HCl-EDTA-buffer. The enzymatic reactions were started by the addition of cellular homogenates. Incubations were stopped after 30 min. by the addition of NaOH. For the extraction of product and non-converted substrate, ethyl acetate containing the internal standard griseofulvin was added to each sample. For the detection of dihydrotestosterone (DHT), the solvent was evaporated and the dried residues were reconstituted in methanol and subsequently subjected to the LC-MS (Surveyor MS Plus HPLC system, Thermo Fisher Scientific).

For the proliferation study, human hair dermal papilla cells (hDPC) were used. Prior to the test, cells were serum starved for 18 h to stop cell proliferation and incubated with the test substance, an extract of hairy roots from *Ocimum Basilicum* for 72 h. Vascular endothelial growth factor (VEGF: Ref. 11343663 from Immunotools) was used as a positive control of cell proliferation. To determine the viability of the cells, a mixture solution of MTT: DMEM (1:2) was added to each well, and cells were incubated for 4 hours at 37 °C in darkness (MTT assay Ref. M2128 from Sigma). Then the reaction was stopped, the supernatant was removed, DMSO added and incubated for 10 minutes. The absorbance was measured at 550 nm (TriStar LB 941, Berthold Technologies, GmbH & Co. KG). As a control, the absorbance of untreated cells was taken (= 100 % viability).

The hair-loss study included 21 volunteers (25 – 67 y, average 51.1 y), 19 females and 2 males, who suffered from mild to moderate hair loss (hair loss > 100 hair strands per daily). During 2 months, the volunteers applied the test fluid containing 1.0 % basil hairy roots extract every day in the evening. To determine the number of lost hair, volunteers collected their lost hair during 3 days (mornings and evenings, only combed hairs in the brush) and placed them in prepared envelopes (hair collections). Hair collections were done on 3 consecutive days and the average of the total counts were taken: day 0 - 3 to determine the baseline, on day 25 – 27 (1 month) and day 53 - 55 (2 months) respectively. Collected hair countings and photographs were taken at the Skin Test Institute (STI).

## Results and Discussion

In our studies we tested a basil hairy roots extract on its activity to inhibit 5 $\alpha$  reductase, the key target enzyme in the inhibition of hair loss. In our study, 5 $\alpha$  reductase I and II isolated from transfected human embryonic kidney cells were used. The inhibitory potency of basil hairy roots extract was measured. As a positive control Finasteride, an approved 5 $\alpha$  reductase inhibitor and synthetic drug to treat baldness in men, was used. The results showed for the basil hairy roots extract a clear concentration dependent inhibition of both isoforms of the enzyme with an IC<sub>50</sub> value of 1.86 mg/mL for 5 $\alpha$  reductase type I and 2.62 mg/mL for the type II isoform (Fig. 3 and 4). This indicates that basil hairy roots extract might be able to reduce hair loss in people with androgenic hair loss.

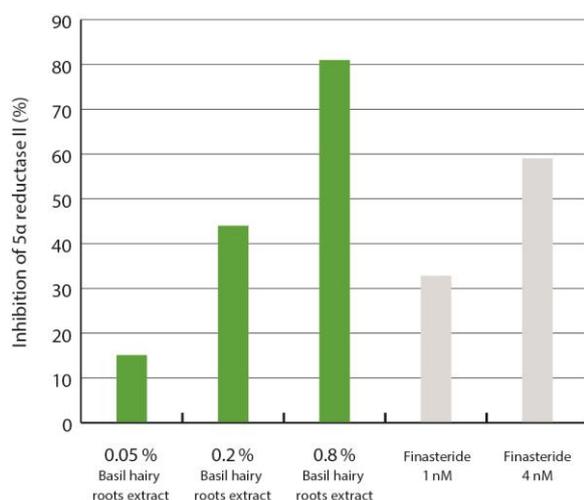


Figure 3: Concentration dependent inhibition of 5 $\alpha$  reductase II by a basil hairy root extract. Positive control: Finasteride.

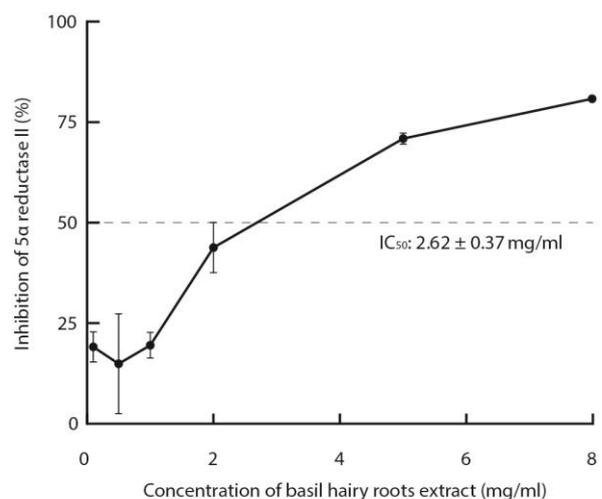


Figure 4: Inhibition of the enzyme 5 $\alpha$  reductase II and determination of the IC<sub>50</sub> value.

Another study was conducted with human dermal papilla cells, the cells that are responsible for hair growth. The cells were serum starved for 18 hours to stop cell proliferation and then treated with the basil hairy roots extract during a period of 72 hours. The effect of the test substance was investigated by the MTT assay to measure the viability of the cells. VEGF (vascular endothelial growth factor) was used as a positive control of cell proliferation. The results showed that the basil hairy roots extract improved the proliferation of the dermal papilla cells by 23% (Fig. 3). This indicates a hair regrowth effect as an increased proliferation of dermal papilla cells can lead to faster growing hair.

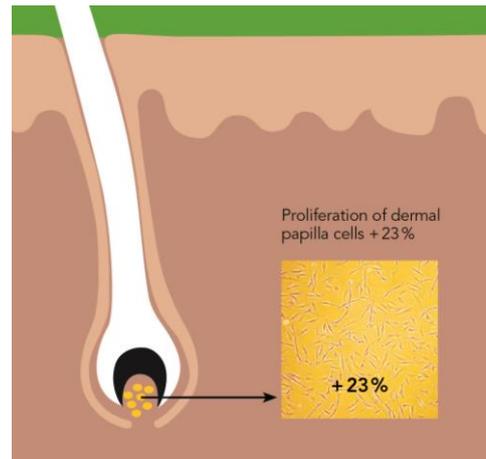


Figure 3: Increased proliferation of dermal papilla cells (23%) after treatment with a basil hairy roots extract.

In a clinical study conducted for two months on 19 women and 2 men with mild to moderate hair loss, basil hairy roots extract showed the capacity to significantly reduce hair loss. Volunteers applied a fluid containing 1% of the basil hairy roots extract every evening over a period of 2 months. Results showed that lost hair dropped by 26 % after the first month and 31 % following the second month (Fig. 4). This suggests that basil hairy roots extract significantly reduces hair loss and thus leads to denser hair.

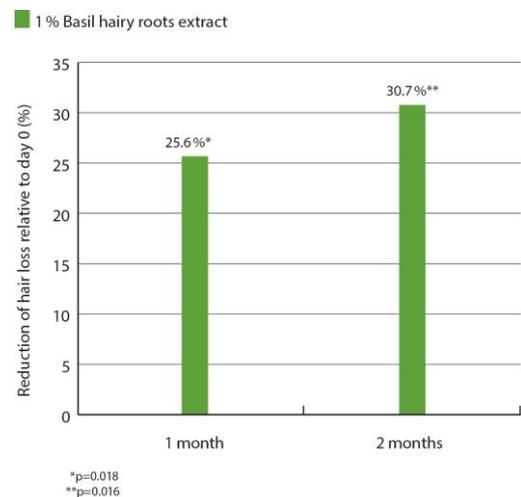


Figure 4: Reduction of hair loss after a treatment with a fluid containing 1% hairy root extract from *Ocimum basilicum*.

## Conclusion

An extract of a hairy root culture of the herb *Ocimum basilicum* was found to inhibit the activity of the hair loss enzyme 5 $\alpha$  reductase II, the key enzyme in androgenic alopecia. Moreover, this extract improved the proliferation of human dermal papilla cells indicating a hair regrowth effect. First results of a clinical study indicate for the basil hairy roots extract a promising potential to treat hair loss as the test substance was able to reduce the number of lost hair by more than 30% after a period of 2 months.

## References

- (1) Immunohistochemical evidence for differential distribution of 5 $\alpha$ -reductase isoenzymes in human skin. Eicher W et al., British Journal of Dermatology.1995 Sept; 133 (3): 371–376.
- (2) Immunohistochemical localization of types 1 and 2 5 $\alpha$ -reductase in human scalp. Bayne EK et al., British Journal of Dermatology: 1999 Sep;141(3):481-91.
- (3) Finasteride, a Type 2 5 $\alpha$ -reductase inhibitor, in the treatment of men with androgenetic alopecia. Kaufman KD and Dawber RP, Expert Opin Investig Drugs. 1999 Apr; 8(4): 403-15.