

Inhibitory Effect of RootBioTec Basilicum Extract on 5 α Reductase I and II

S-490

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Introduction

5 α reductase is the key target enzyme in the inhibition of hair loss. 5 α reductase catalyses the reduction of testosterone into the more potent androgen dihydrotestosterone (DHT).

5 α reductase type II has been detected in scalp hair follicles. Balding scalps contain increased 5 α reductase type II activity and dihydrotestosterone (DHT) levels. 5 α reductase type I which also metabolizes testosterone to DHT, is the principal isoenzyme in sebaceous and sweat glands.

For the following study, human 5 α reductase I and II were used.

The inhibitory potency of RootBioTec Basilicum extract was measured and compared to Finasteride, an approved 5 α reductase inhibitor.

Test products

- RootBioTec Basilicum extract 0.1 - 8 mg/mL (Ocimum Basilicum hairy root extract)

Reference product

- Finasterid (5 α reductase inhibitor)

Test parameters

- 5 α reductase I and II activity
- IC₅₀ Value: The half maximal inhibitory concentration. Represents the concentration of a product that is required for 50 % inhibition in vitro.

Assay

Preparation of 5 α reductase type I and II containing cell fractions:

HEK I and II cells were cultivated in DMEM. For the cell-free in vitro assay HEK I and II cells were harvested, freed from culture medium by centrifugation and resuspended in a homogenate-buffer. By freezing at -196°C and thawing on ice, the cells were solubilised. After an incubation period of 30 min. at 4°C with DNase in MgCl₂ the obtained homogenate was centrifuged. The pellet was resuspended in homogenate buffer and was centrifuged again.

The homogenate pellet was detached from the tube bottom with homogenate buffer and resuspended by ultrasonic-

mixing. Protein content was quantified using the commercially available RotiQuant® reagent. The fractionated cell suspension was stored at -80°C, for the 5 α reductase assay.

5 α reductase assay:

Incubations were performed at 37°C in a Tris HCl-EDTA-buffer (assay buffer) containing NADPH, androstenedione, cell homogenate and RootBioTec Basilicum extract. Finasteride, used as a positive control, was dissolved in ethanol and further diluted in Tris HCl-EDTA-buffer. Solvent treated controls were handled the same way and contained ethanol. 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 were added to each sample.

LC-MS:

The solvent was evaporated and the dried residues were reconstituted in methanol and subsequently subjected to the LC-MS.

Material and Methods

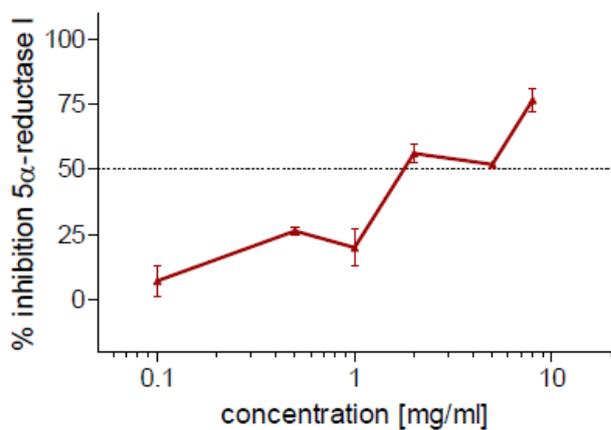
- LC-MS/MS instrument: Surveyor MS Plus HPLC system (Thermo Fisher Scientific).
- 5 α reductase assay

Result

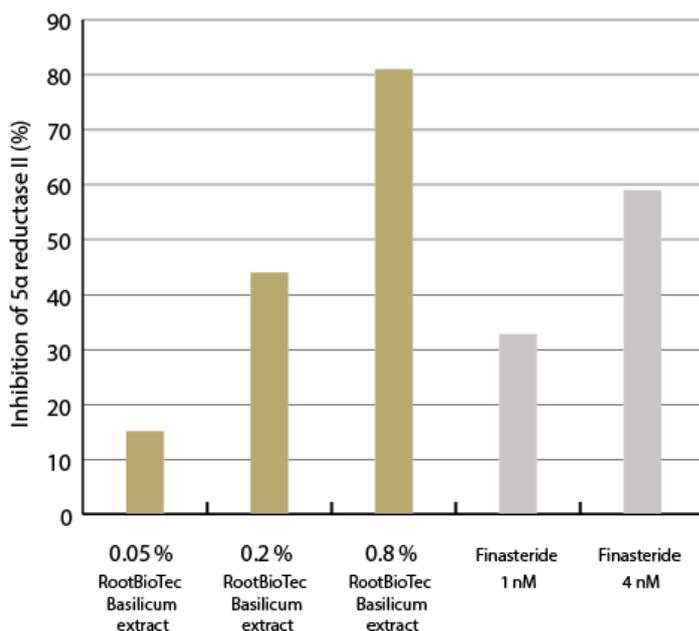
The results with the test product RootBioTec Basilicum extract showed a clearly concentration dependent inhibition of 5 α – reductase I and II with an IC₅₀ value of 1.84 for 5 α – reductase type I and 2.62 for 5 α – reductase type II.

5 α -Reductase I Inhibition by RootBioTec Basilicum Extract

Test concentration	Mean inhibition (%), n=2
8.0 mg/ml	76.6
5.0 mg/ml	51.9
2.0 mg/ml	56.1
1.0 mg/ml	19.9
0.5 mg/ml	26.3
0.1 mg/ml	7.1
Finasteride 1250 nM	66.6
Finasteride 250 nM	36.2



5 α -Reductase II Inhibition by RootBioTec Basilicum Extract



Test concentration	Mean inhibition (%), n=2
8.0 mg/ml	80.8
5.0 mg/ml	70.9
2.0 mg/ml	43.8
1.0 mg/ml	19.5
0.5 mg/ml	14.9
0.1 mg/ml	19.1
Finasteride 4 nM	58.8
Finasteride 1 nM	32.6

