WEB325 - Flavonoids from *N.benthamiana*

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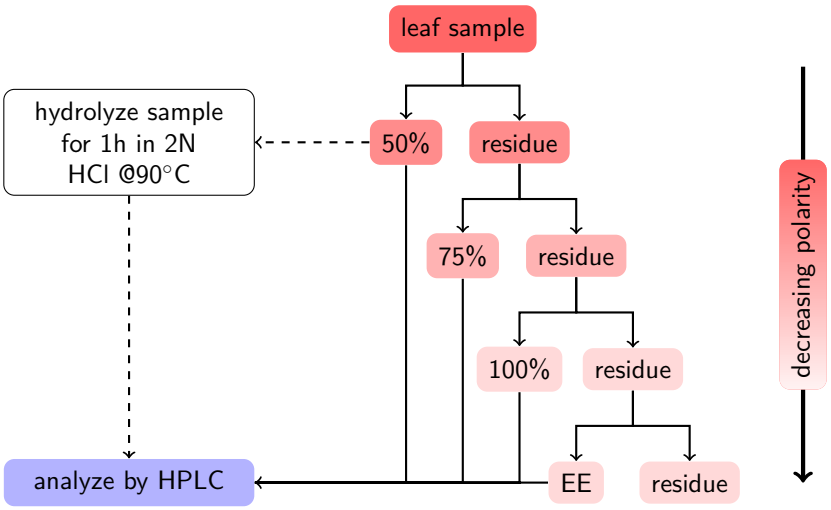

Figure 1: Stepwise extraction of *N. benthamiana* leaves by decreasing polarity. Supernatant fractions: **X%** – *X*% methanol, **EE** – ethyl acetate.

1 Extraction test of *N.benthamiana* leaves

- froze, grinded and lyophilized residual leaves from WEB313
- average weight loss of WEB313 samples was 87.5% → use 6.25 mg (12.5% of 50 mg, which is double the amount used in the master thesis of Kristin König) dried plant material for extraction with 500μl solvent

1. extract with 500 μl 1 mM ascorbic acid, 0.2 % formic acid in 50 % (v/v) MeOH
   → 30 s vortex, 10 min rotary shaker, 30 s vortex
   → centrifuge at 4°C 10.000g for 10 min
   → collect supernatant
   → centrifuge at 4°C 10.000g for 10 min
   → collect 400μl supernatant

2. extract with 500 μl 1 mM ascorbic acid, 0.2 % formic acid in 75 % (v/v) MeOH
   $\rightarrow$ like **1.**

3. extract with 500 μl 1 mM ascorbic acid, 0.2 % formic acid in MeOH
   $\rightarrow$ like **1.**

4. extract with 500 μl 2 % formic acid in ethyl acetate
   $\rightarrow$ like **1.**
   $\rightarrow$ dry in speedvac & resuspend in 400μl 1 mM ascorbic acid, 0.2 % formic acid in MeOH

1.1 Hydrolysis of glycosylated compounds

To analyse the aglycone flavonoid content in the 50% methanolic sample, the glycosylated flavonoids needed to be hydrolyzed. Therefore 300 μl of each of the 50% MeOH fractions were hydrolyzed, by adding 300 μl of 4 N methanolic HCl and heating to 90°C for 1 hour.

The resulting solution was evaporated in a *SpeedVac* set to 60°C and the residue was resuspended in methanol containing 1 mM ascorbic acid and 0.2% formic acid. The resulting solution was centrifuged at 10.000 x g and 4°C for 10 minutes. The supernatant wa analyzed by HPLC.

Attention: Some of the tubes sprung open during heating.