

PLANT IN VITRO CULTURES AS SOURCE OF COLLAGEN CROSS-LINKING SUBSTANCES

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Introduction

Plants can be used as alternative source of potential protein cross-linking metabolites, so-called tanning agents¹. These metabolites are relevant compounds for producers of collagen based biomaterials in the field of medicine technology and cosmetics as well as food and leather production. Cross-linking of collagen is performed almost exclusively using substances which bear a toxic potential or are produced on the basis of fossil fuels such as glutaraldehyde, isothiocyanates or chromium salts. Iridoids and Secoiridoids are secondary plant metabolites showing a less toxic behavior but similar cross-linking abilities compared to the common tanning agents².

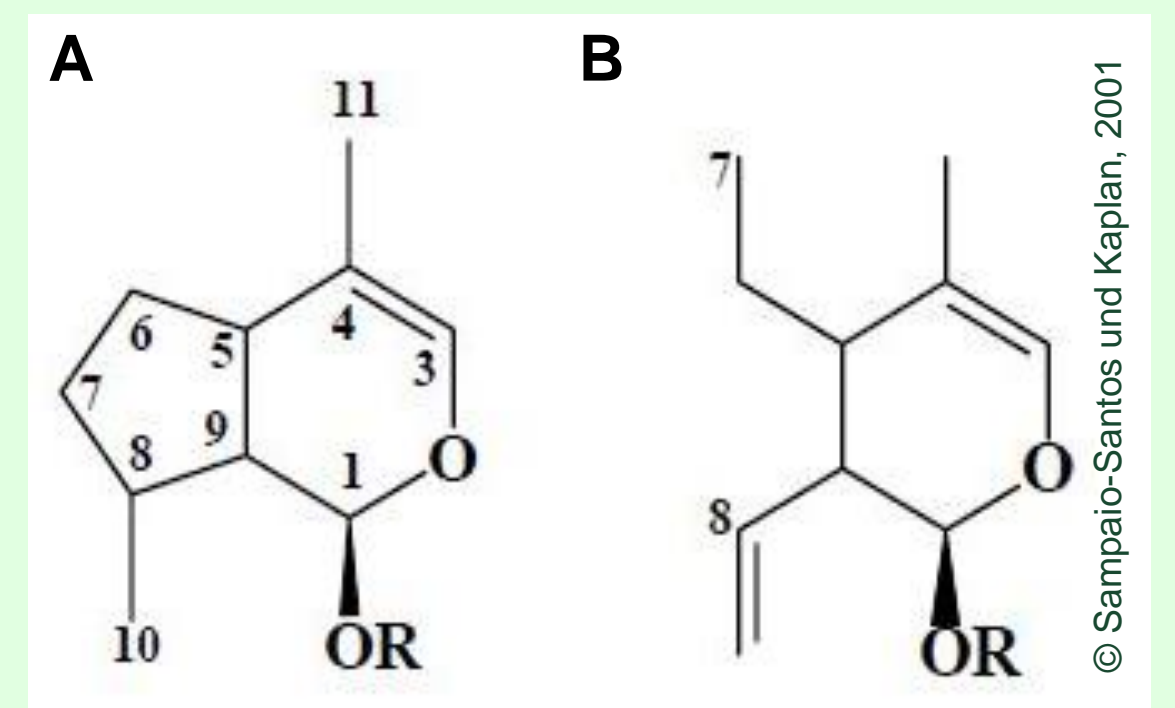


Fig. 1: Iridoids (A) and Secoiridoids (B) are mono-terpenoids produced by plants as a defense against herbivores and infection by microorganism.

Screening for plants containing tanning agents

- examination of plant extracts with a SDS-PAGE based cross-linking test
- cross-linking of the collagen molecules was verified by SDS-PAGE
- promising candidates for further experiments shown in **Fig. 2**
- HPLC analysis of plant extracts to identify (Seco)-Iridoids (**Tab. 1**)

Tab. 1: Secoiridoid content of leaf extracts in ethanol/H₂O (1:1), percentage refers to the total dry extract.

	Secoiridoids in leaves (content in %)
<i>G. lutea</i>	Gentiopicrosid (5.7 ± 0,1 %), Amarogentin (0.3%)
<i>C. erythraea</i>	Swertiamarin (11.8 ± 2,3 %), Gentiopicrosid (0.9 ± 0,1 %), Swerosid (1.3 ± 0,73 %)
<i>O. europaea</i>	Oleuropein (10.9 ± 0,6 %)
<i>S. vulgaris</i>	Oleuropein (6.4 ± 0,6 %)

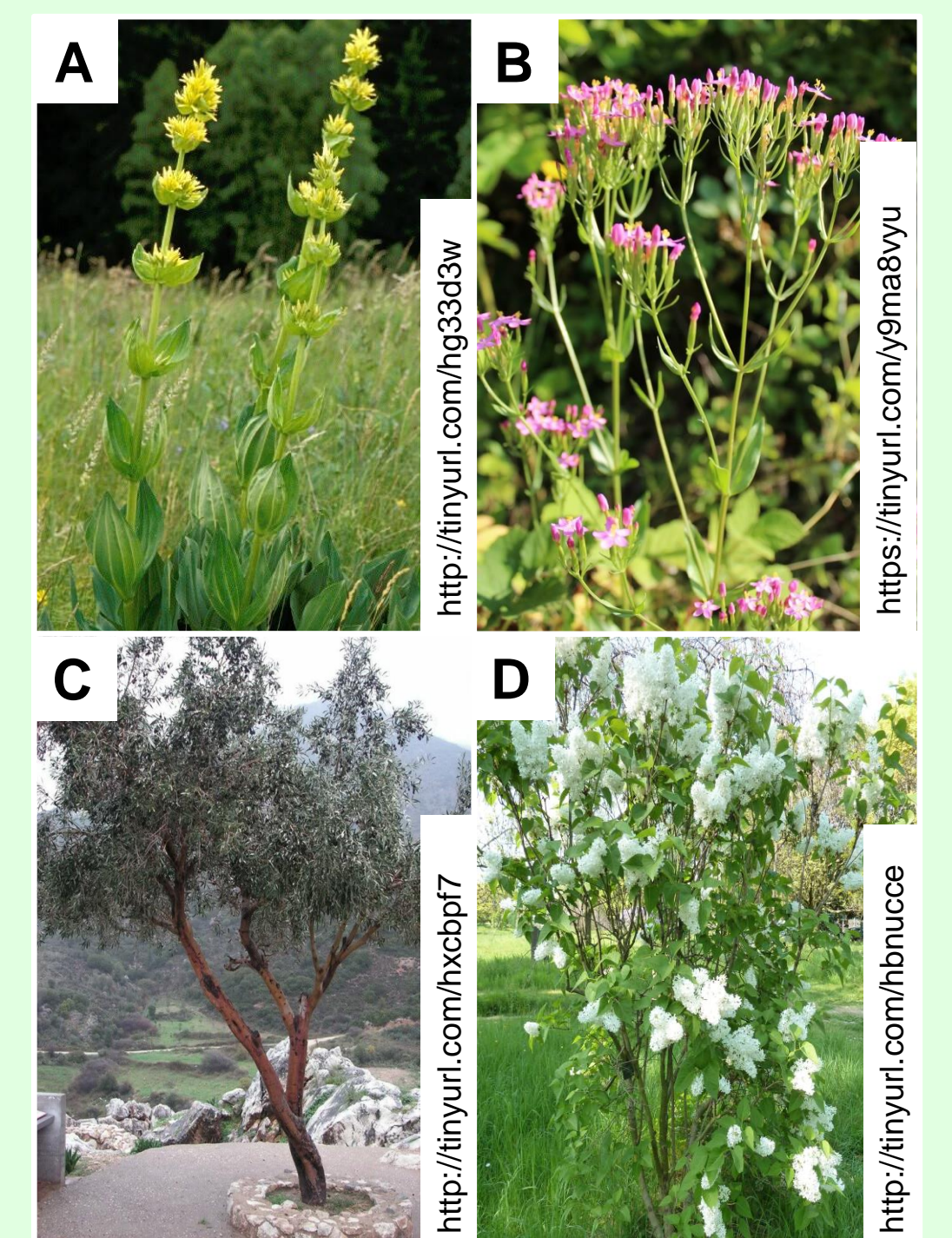
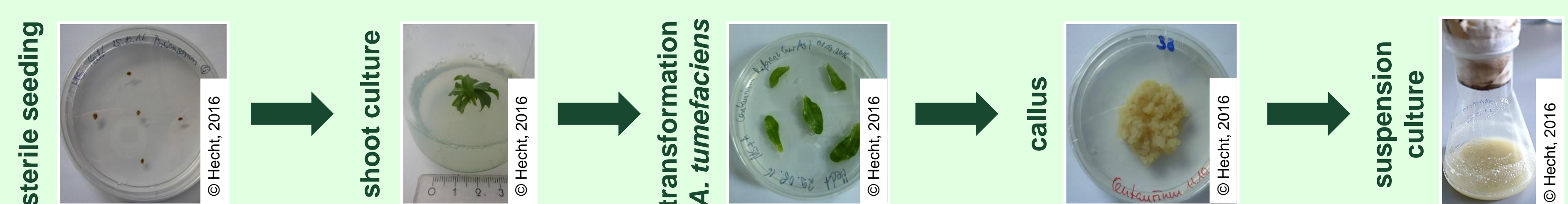


Fig. 2: Promising candidates containing tanning agents: *Gentiana lutea* (A), *Centaurea erythraea* (B), *Olea europaea* (C) and *Syringa vulgaris* (D).

Initiation of plant cell suspension cultures



Current state of work

- shoot cultures of *G. lutea*, *C. erythraea*, *O. europaea* and *S. vulgaris*
- callus cultures of *C. erythraea* and *S. vulgaris*
- Cross-linking tests via SDS-PAGE *C. erythraea* shoots and callus together with *S. vulgaris* (shown in **Fig. 3**)

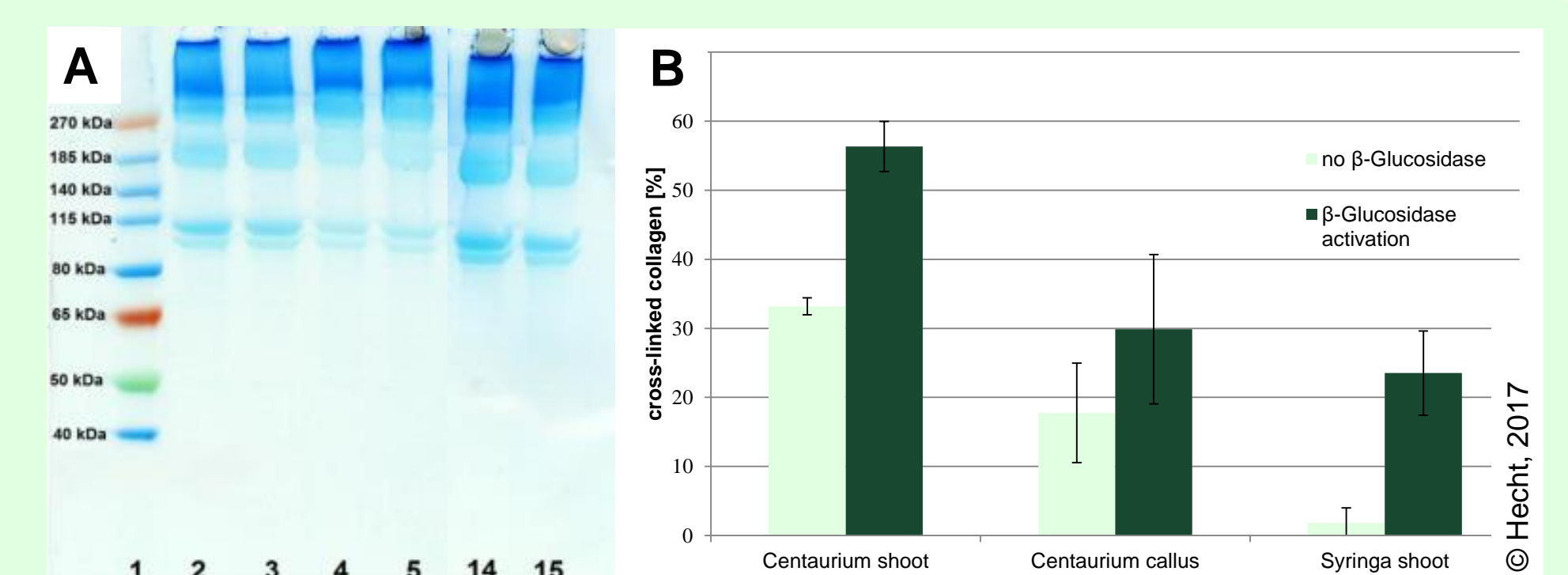
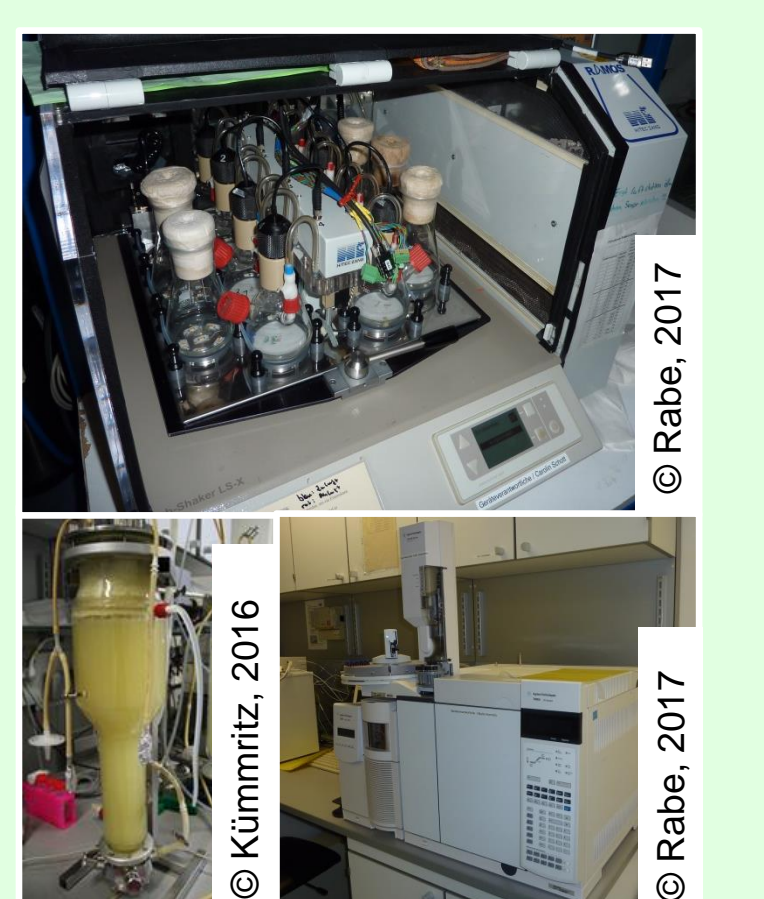


Fig. 3: Results of cross-linking test for acid solubilised collagen treated with *C. erythraea* and *S. vulgaris* extract. **A:** 1) protein ladder, 2-3) extract, 4-5) extract + β -Glucosidase, 14-15) acid solubilised collagen. **B:** Amount of cross-linked collagen.

Further steps towards scale up

- Respiration Activity MOnitoring System[®] (RAMOS[®]) to characterise cell suspension cultures
- application of different growth conditions and media to increase (Seco)-Iridoids yields
- HPLC and GCMS measurements in order to identify and quantify (Seco)-Iridoids
- up scale and optimisation of downstream processes



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References

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