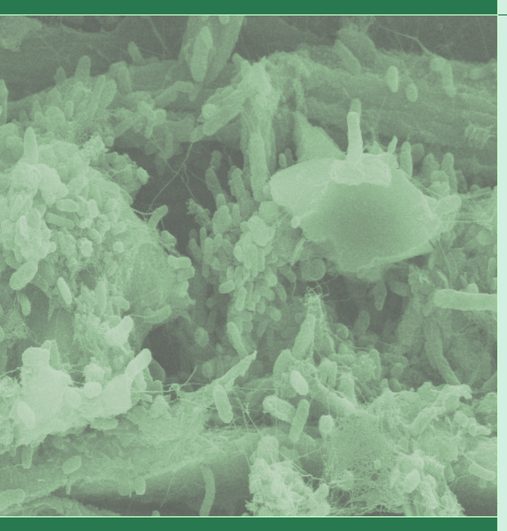
Screening methods for pharmaceutical relevant triterpenoids

S. Schulz[1], Ch. Haas[1], D. Leipold[2], K. Muffler[2], R. Ulber[2], A.I. Pavlov[3,4], T. Bley[1], J. Steingroewer[1]

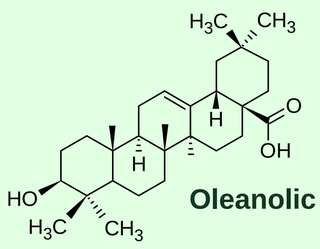
[1]Institute of Food Technology and Bioprocess Engineering, Technische Universität Dresden, 01062 Dresden, Germany
[2]Institute of Bioprocess Engineering, University of Kaiserslautern, 67663 Kaiserslautern, Germany
[3]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 4000 Plovdiv, Bulgaria
[4]Department of Organic Chemistry and Microbiology, University of Food Technologies, 4000 Plovdiv, Bulgaria



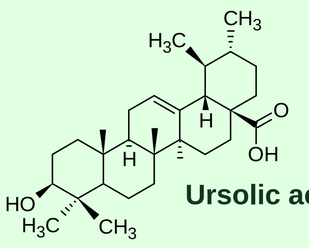
Introduction and Aims:

Plant cell, tissue and organ cultures are a prospective alternative for bioactive natural substances production. Therefore screening and selection of high productive cultures are required for yield enhancement. In order to perform an efficient screening, rapid, selective and sensitive methods are necessary to be developed.

A previous screening of ethanolic extracts of *Salvia* suspension cultures (*S. officinalis* (Fig.2), *S. triloba* and *S. virgata*) by HPLC-UV measurement for the production of oleanolic and ursolic acid indicated a production of further compounds of pharmaceutical interest. For the screening of secondary metabolite production callus extracts of these three *Salvia* species have been investigated by GC-MS and compared to the metabolite profile of the plants.



Oleanolic acid (OA)



Ursolic acid (UA)

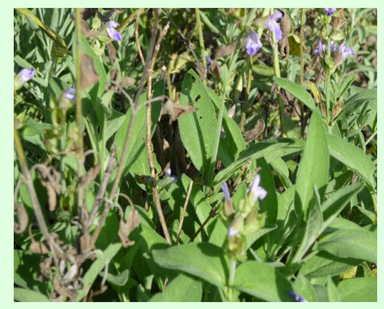


Fig.1 *Salvia officinalis*

Fig. 2 Callus from *Salvia officinalis*

GC-MS Analysis:

Agilent GC 7890 MSD 5975C inert (EI 70 eV)

HP-5ms column (30m x 250µm x 0.25µm)

He-flow 1ml/min, injection 1µl, split: 1:50

Oven: 80℃ (1min) with 10℃/min to 250℃ then 2℃/min to 300°C (15min)

Internal Standard cholesterole (c=104µg/ml)

MSD simoultanous sim-scan-mode, scan 50-550m/z

Data analysis:

Comparison of measured mass spectra and RI-indices (C7-C40-Standard) after deconvolution with AMDIS 2.69 Software with Wiley 08, CSB DB Golm and Golm Metabolome Database

Tab. 1 Identified compounds and their biological and pharmaceutical properties

No.	Compound	Some biological and pharmacological properties
1	Trans-ferulic acid	antioxidative, anti-carcinogenic, antimicrobial
2	Trans-caffeic acid	antioxidative, anti-inflammatory, anti-carcinogenic
3	Stigmastan-3,5-diene	antioxidative, inhibits cholesterol absorption
4	β-Sitosterol	antioxidative, inhibits cholesterol absorption and proliferation of human leukemia cells
5	Rosmarinic acid	adstringent, antioxidative, antiplatelet, antibacterial, antiviral, antimutagen
6	Oleanolic acid (OA)	anti-inflammatory, antibacterial, hepatoprotective
7	Ursolic acid (UA)	anti-inflammatory, antibacterial, anticancerogen, antifungal, anti-tumor
8	3β-Acetoxy-oleanolic acid	anti-carcinogenic

Results:

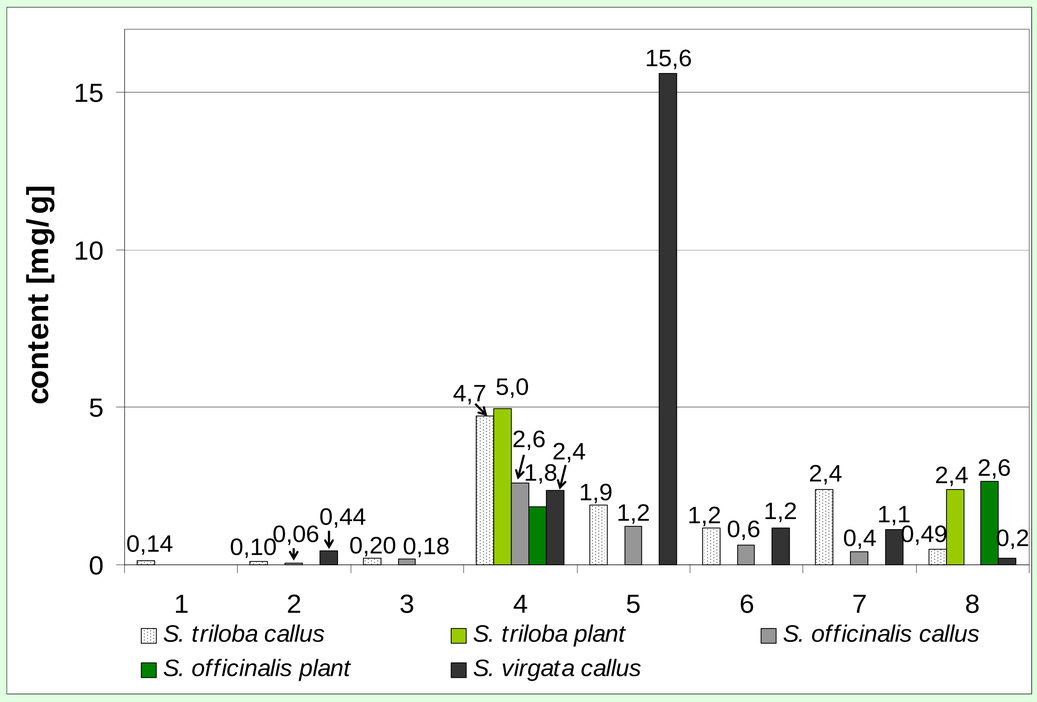


Fig. 3 Results of GC-MS screening of suspension cultures of Salvia sp., different cultivation phases compared with metabolites determined in plant biomass (numbering of the compounds see Tab. 1)

→ *S. virgata* suspension culture showed high production of rosmarinic acid (esp. growth phase)

→ β-Sitosterol mainly produced by *S. triloba*, as well as *S. officinalis* and less by *S. virgata* suspension culture (for all approximately constant during cultivation)

→ 3β-Acetoxy-oleanolic acid mainly produced by *S. officinalis* suspension culture as also observed in the plant material

Determination of OA and UA in *Salvia* leaves as reference, clean-up by anion exchange chromatography (recovery about 100%):

Tab. 2 Determined amount of OA and UA in leaves of *Salvia* sp.

Species	OA [mg/gDW]	UA [mg/gDW]
S. triloba	10.8	31.1
S. officinalis	7.5	22.4
S. virgata	5.5	2.8

Conclusion & future prospects:

The analysed callus extracts of the three *Salvia* species indicated the productivity of further pharmaceutical relevant metabolites: beside OA and UA also β-Sitosterol and rosmarinic acid occur in high amounts, which deviates from the plants.

The productivity of OA and UA in *Salvia* suspension cultures is about 10% less compared with the plants and should be enhanced by cultivation optimisation and elicitation (see poster P 40.11, C. Haas).

Further experiments for the isolation and purification of OA and UA from the *in-vitro* cultures on a larger scale will be performed.

contact:
Dipl. LM.Chem. Sibylle Schulz
Phone: +49 351/463 39042
Sibylle.Schulz@tu-dresden.de

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