Analysis of oleanolic and ursolic acid in Salvia species and plant cell cultures

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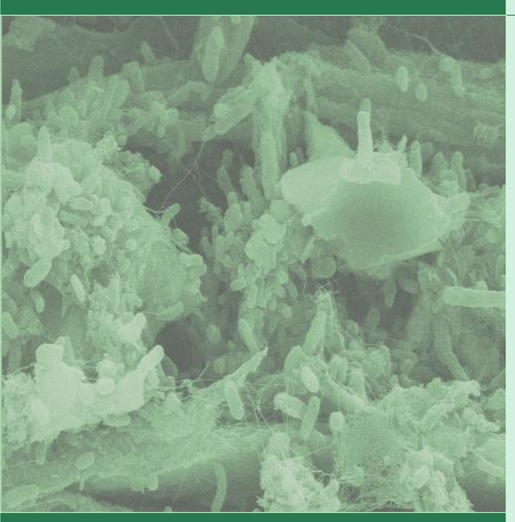
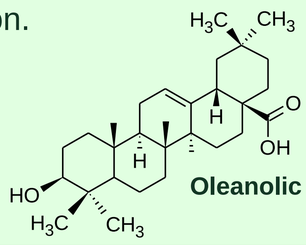


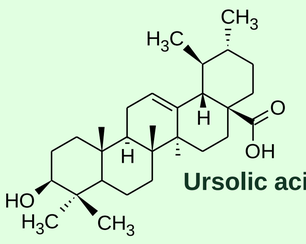
Introduction and Aims:

Salvia sp. as a widespread kitchen herb exhibits a variety of biological active compounds that emphasizes its pharmaceutical importance. Two of these secondary metabolits are the triterpenic acids oleanolic and ursolic acid. They have been reported to possess anti-inflammatory, anti-tumour and anti-HIV effects and are commercially used in cosmetics and health products. Due to the similarity of chemical structures and polarities of oleanolic and ursolic acid a separation which is essential for an accurate, quantitative determination and the supply of the pharmaceutical product, is difficult and has been widely investigated [1], [2].

In this study a screening method for both triterpenic acids via thin-layer chromatography (TLC) as a sum parameter was established and applied to ethanolic extracts of plant and plant cell cultures in order to reveal the productivity of these cultures. Amounts of the compounds were measured by HPLC-UV for quantification.



Oleanolic acid (OA)



Ursolic acid (UA)

Fig.1 *Salvia officinales*

Fig. 2 Callus from *Salvia officinales*

Materials and Methods:

Sample preparation:

- 0.1 g lyophilized plant or culture material grinded and extracted with 100% ethanol in ultrasonic bath (15 min at 45 °C),
- Plant material preextracted with petrolether,
- Separation via centrifugation (10 min at 5000 rpm),
- Supernatant used directly for TLC-analysis, filtered through a 0.2 µm PTFE-membrane filter.

TLC:

TLC-plate silica gel 60 (Merck, Darmstadt),

Preconditioned with methanol

Mobile phase:

Methanol/acetone/acetonitrile/toluol (10/10/10/30, v/v/v/v)

Sample volume 10 µl

Standards 5 µl 0.1 mg/ml OA/UA

Detection: anisaldehyde reagent;

UV at 366 nm

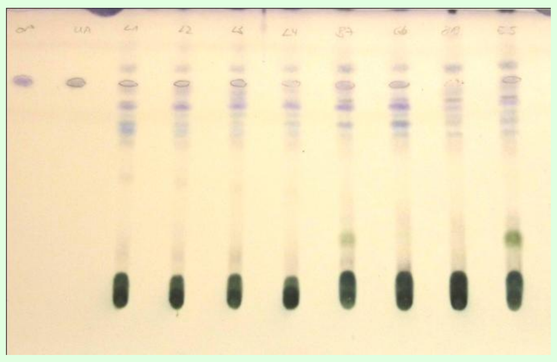


Fig. 3 TLC-chromatogram of callus-extracts

HPLC

Hewlett Packard 1050 series

Discovery HS C18 (5 µm 25 cm x 4,6 mm) column at 20 °C

Mobile phase:

Methanol/0.1% formacid (92/8, v/v)

Injection 20 µl

Flow 0.5 mg/ml

UV detection at 210 nm

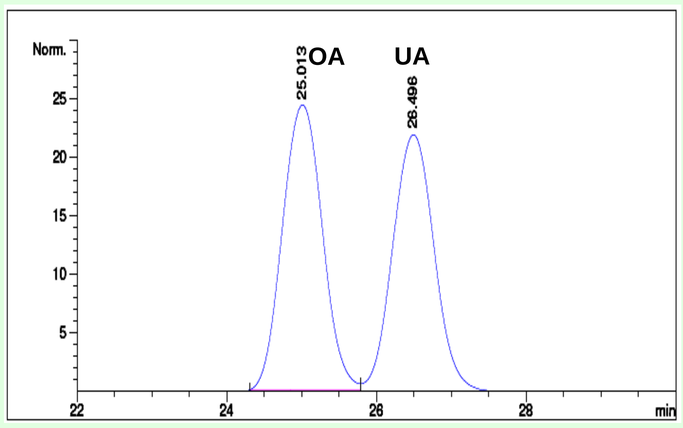


Fig. 4 HPLC/UV chromatogram OA/UA Standard 50 µg/ml

Tab. 1 Method validation HPLC

Standard	Regression equations[a]	Correlation coefficient (R^2)[a]	RSD [µg/ml][b]	LOD [µg/ml][b]	LOQ [µg/ml][b]
Oleanolic acid	y = 18.463x + 1.041	0.99993	4.64	0.32	1.05
Ursolic acid	y = 17.128 + 3.75e-1	0.99995	5.22	0.36	1.18

[a]7point-calibration curves at concentrations of 2.5 – 250 µg/ml
[b]determined by analysing the 2.5 µg/ml standards (n = 10)

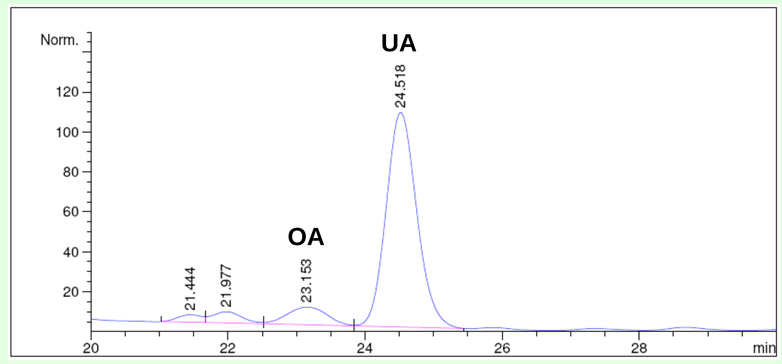


Fig. 5 HPLC-Chromatogram callus extract *Salvia officinales*

Results:

TLC-Method

- Simple method for screening different extracts,
- Estimated LOD 0.05 µg for OA, 0.13 µg for UA per spot,
- Screening 102 callus extracts (see poster of Christiane Haas): predicted production was confirmed by HPLC,
- Mean R_f value 0.76 for oleanolic and ursolic acid.

HPLC-Method

- Good separation of the isomers obtained,
- Good linearity over the concentration range 2.5-250 µg/ml (R^2 values exceeding 0.999),
- Precision RSD about 5% at lowest concentration of calibration,
- LOD 0.32 µg/ml OA; 0.36 µg/ml UA,
- LOQ 1.05 µg/ml OA; 1.18µg/ml UA.

The application of these methods provided an insight into the variability of the production of the *Salvia* plants and plant cell cultures. Non or less productive cultures could be segregated. Accordingly this enables the control of the biological process involved.

Future prospects:

Measurements of the extracts for identification of the triterpenic acids and other compounds of pharmaceutical interest will be performed by GC-MS after derivatization.

These methods will be useful for the optimization of culture parameters to enhance the productivity of the cultures and consequently the yield.

References:

[1] Lee, M.K. et al., 2009. Development of a validated liquid chromatographic method for the quality control of Prunellae Spica: Determination of triterpenic acids. *Analytica Chimica Acta*, 633(2), 271-277.
[2] Leipold, D. et al., 2010. Biosynthesis of ursolic acid derivatives by microbial metabolism of ursolic acid with Nocardia sp. strains—Proposal of new biosynthetic pathways. *Process Biochemistry*, 45(7), 1043-1051.

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