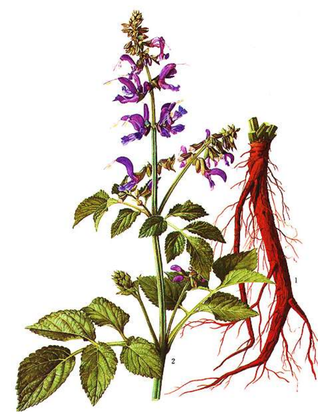
HPTLC-EDA-HRMS and PLC-NMR spectroscopy for structural elucidation of active compounds in *Salvia miltiorrhiza*

E. Azadniya, G. Morlock

Justus Liebig University Giessen, Germany

JLU Study life Explore the world



Highlights

- The proven activities of *Salvia miltiorrhiza* root (Danshen) as AChE inhibitor, DPPH˙ scavenger and antimicrobial against Gram-positive and Gram-negative bacteria explain its worldwide acceptance as multipotent natural product
- Two-step HPTLC method developed with a low acid content for bioprofiling of polar and apolar extracts of *Salvia miltiorrhiza* root: 1st development with toluene - chloroform - ethyl acetate - methanol - formic acid 4:6:8:1:1 up to 45 mm and 2nd with petroleum ether - ethyl acetate - cyclohexane 5:2.2:2.8 up to 85 mm
- For bioprofiling, investigation of liquid-liquid extractions for polar and nonpolar Danshen fractions, including different solvents
- Comprehensive bioprofiling via hyphenation to *B. subtilis*, *A. fischeri,* acetylcholinesterase (AChE) and DPPH˙ (bio)assays, followed by characterization of bioactive compounds by HESI-HRMS using an elution-head based TLC-MS interface
- Fast and cost-efficient structure elucidation of a apolar unknown bioactive zone by PLC-NMR spectroscopy to be 1,2-dihydrotanshinone and methylenetanshinquinone co-eluting in the ratio 2:1

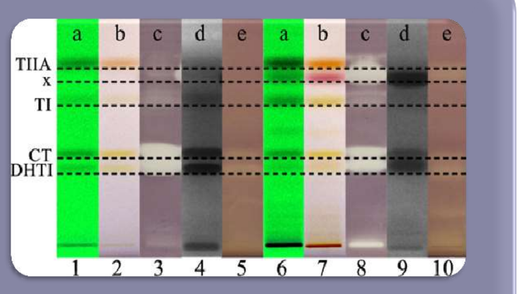


Fig. 2 Documentation at UV 254 nm (a), white light illumination (b), after *B. subtilis bioassay* (c), *A. fischeri* bioassay (d), AChE assay (e); tracks 1-5 standards of tanshinones, tracks 6-10 extract via ethanol – dichloromethane 4:1

Apolar

Bioprofiling via HPTLC–EDA

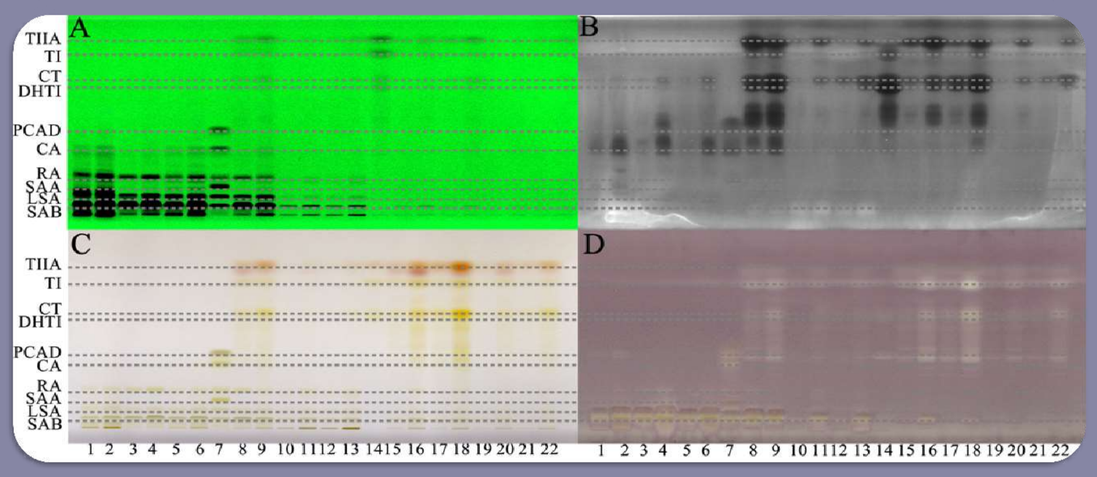


Fig. 1 HPTLC-EDA of different extraction solvents: methanol (tracks 10-13), ethyl acetate extract of acidified water fraction (1, 2), same plus salt and acetone (3-6), ethanol (19-22) and ethanol – dichloromethane 4:1 (8, 9, 15-18) along with standard mixture (7, 14) at UV 254 nm (A), after *A. fischeri* bioassay, under white light illumination (C) and after *B. subtilis* bioassay (D)

Polar

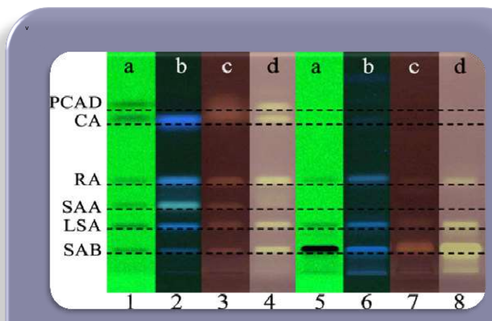


Fig. 4 Documentation at UV 254 nm (a), UV 366 nm (b), after AChE *assay* (c), DPPH* assay (d); tracks 1-4 standards of phenolics and tracks 5-8 extracts via methanol

HPTLC-HRMS

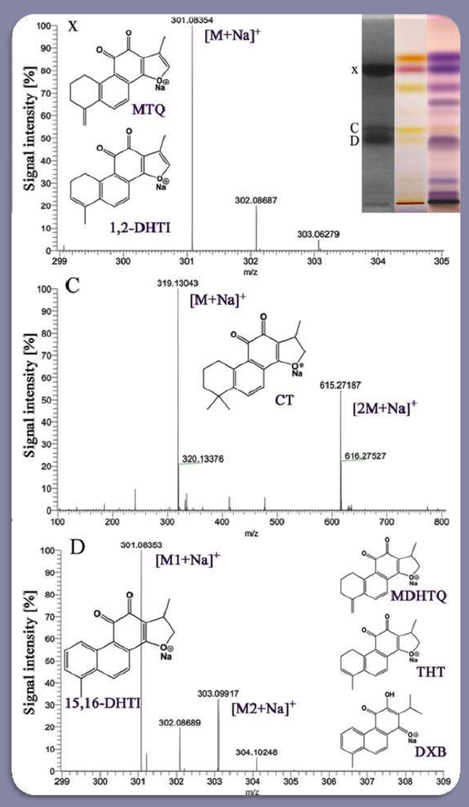


Fig. 3 HPTLC-ESI⁻-HRMS of apolar bioactive compounds (X, C, D) of Danshen

PLC-NMR spectroscopy workflow

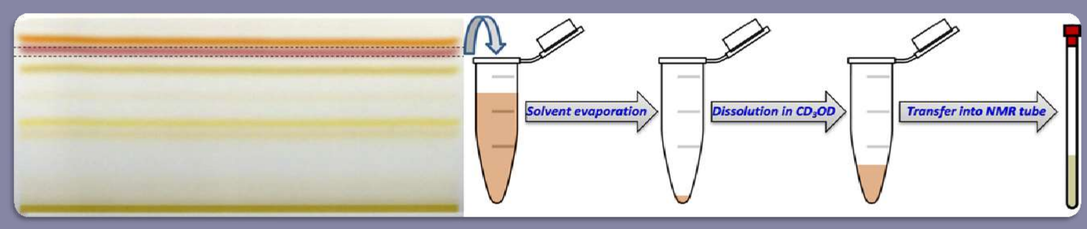


Fig. 6 Danshen (2 mL, 200 mg/mL) was applied as band 185 mm × 3 mm on the PLC plate (20 × 10 cm) and developed up to 10 cm; the active zone was scraped off and extracted 3 times with 2 mL methanol; after evaporation, the residue was dissolved in deuterated methanol, transferred into a 5-mm NMR tube and measured with the NMR spectrometer AV 600 (Bruker)

Structure elucidation of unknown bioactive zone

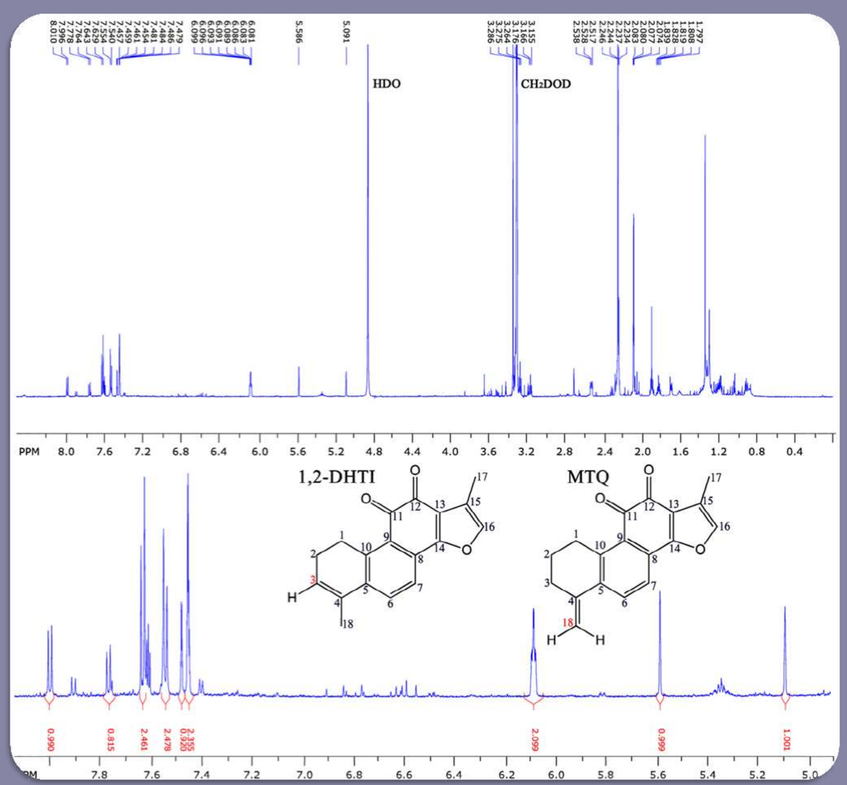


Fig. 7 ^{1}H-NMR spectrum of unknown compound "x" led to the combination of 1,2-DHTI and MTQ

HPTLC-HRMS

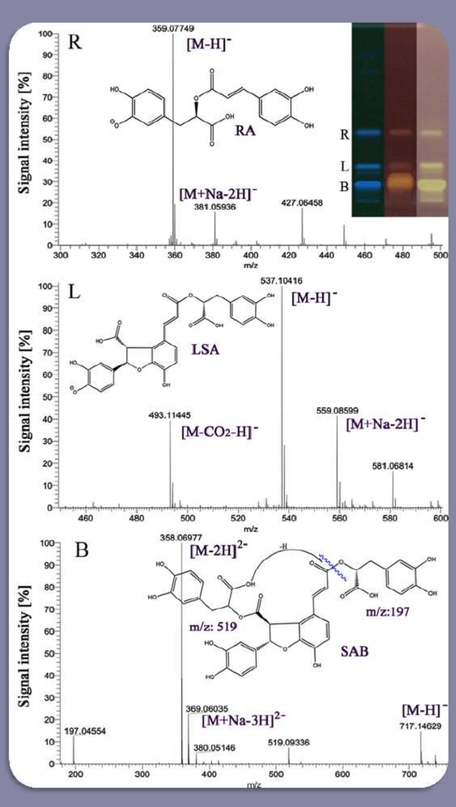


Fig. 5 HPTLC-ESI⁻-HRMS of polar bioactive compounds (R, L, B) of Danshen

Acknowledgement

Thank is owed to Dr. H. Hausmann, Institute of Organic Chemistry, JLU Giessen, for NMR measurements, Merck, Darmstadt, Germany, for plates and Phytolab, Vestenbergsgreuth, Germany for phenolics.

IFZ Interdisziplinäres Forschungszentrum Giessen Research Centre for BioSystems, Land Use and Nutrition

Food Science, Justus Liebig University Giessen, Institute of Nutritional Science, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany