Effect-directed analysis of *Salvia officinalis*

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Introduction

The chemical constituents of *Salvia* have been investigated for more than 60 years. Pharmacological studies indicated that members of this plant family contain a variety of bioactive compounds – phenolics, flavonoids and triterpenoids. [1] Since *Salvia officinalis* and its medicinal products are also pharmaceutically used, an easy and non-targeted analysis method is essential to ensure the efficiency and stability of commercial products. Hyphenation of HPTLC with bioassays, microchemical detection and MS enabled first non-targeted detection of bioactive compounds from plant extracts and then their highly targeted identification. In this study, hyphenated HPTLC-UV/FLD/Vis-EDA-MS was used for fingerprinting of polar and nonpolar bioactive compounds of *Salvia officinalis*.

Results and discussion

Microchemical detection

HPTLC plates silica gel 60 with ethanolic *S. officinalis* extracts were separately developed with a polar and apolar mobile phase (Fig. 1). In order to comprehensively characterise unknown components of *S. officinalis*, the obtained chromatograms were subjected to different microchemical reagents. Multi-detection of bioactive zones was performed (Fig. 1, dashed line based on hR_F values). The universal fingerprint of *S. officinalis* extracts was visualized with anisaldehyde sulfuric acid reagent (Fig. 1: 7). Fast blue B salt reagent (Fig. 1: 2) and 2,4-dinitrophenylhydrazine reagent (Fig. 1: 3) enabled the visualization of phenolic and carbonyl compounds, respectively. Compounds with vicinal hydroxyl groups like flavonoids were detected by Neu's reagent (Fig. 1: 5). With the DPPH* reagent, the ethanolic *Salvia* extract showed many strong antioxidative compounds, which were polar to middle-polar based on the chromatographic fingerprint.

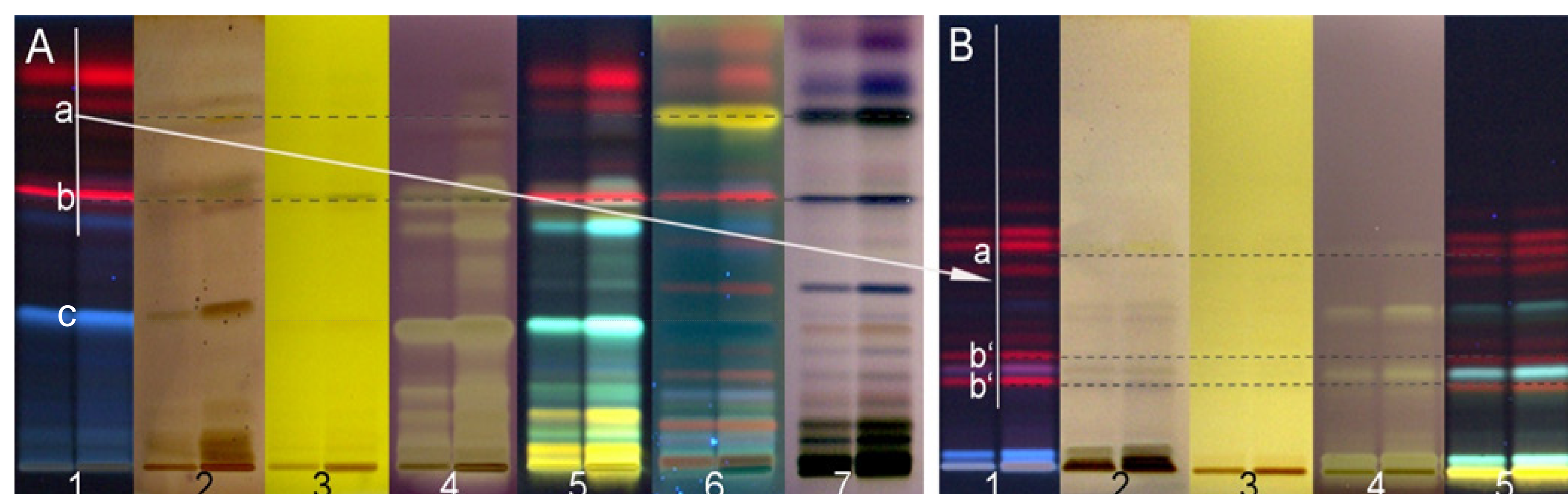

Fig. 1 Fingerprints of *S. officinalis* (A: 2 and 5 µL and B: 1.6 and 2.4 µL) detected (1) at UV 366, (2) after fast blue B salt reagent, (3) after 2,4-dinitrophenylhydrazine reagent, (4) after DPPH* reagent, (5) after Neu's reagent and (6 and 7) after anisaldehyde sulfuric acid reagent; documented (1,5 and 6) at UV 366 nm and (2-4 and 7) under white light illumination.

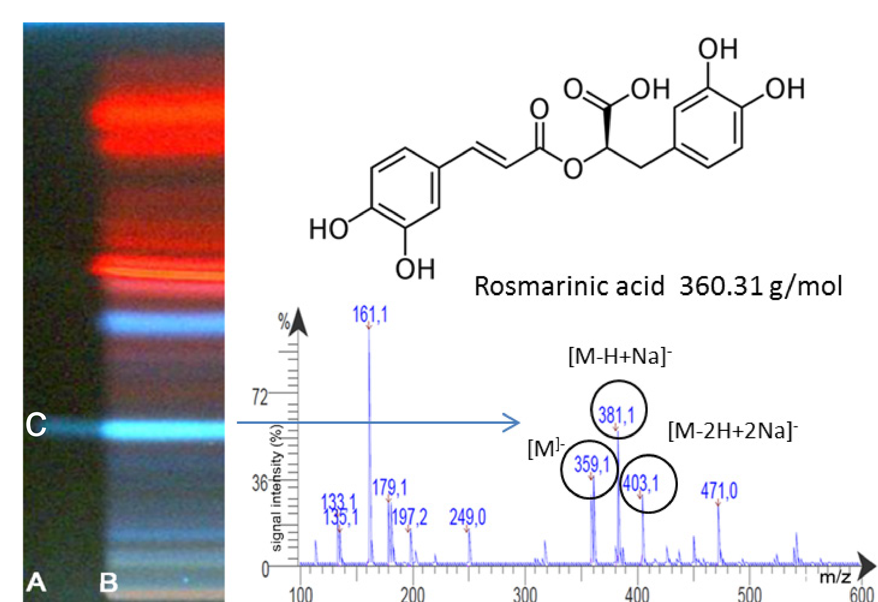

Fig. 2 Identification of rosmarinic acid (c) in *S. officinalis* (B) by oversprayed application of standard solution (A) and HPTLC-MS.

Identification of rosmarinic acid

One compound of major antioxidative response was rosmarinic acid (C). This was proven by oversprayed application and separation of the added standard solution within the plant matrix (Fig. 2, A and B). The assignment was confirmed by HPTLC-ESI⁻-MS, which showed the molecular ion at *m/z* 359 and its sodium adduct at *m/z* 381 (Fig. 2, C).

Bioactivity of Salvia

Zones a and b showed a strong inhibition for cholinesterase and for α-glucosidase [2] as well as an antimicrobial activity against *Bacillus subtilis* (Fig. 3). The hR_F value of luteolin fit to the hR_F value of the bioactive zone b. For its confirmation, HPTLC-MS will be employed. Zone c showed a strong α- and β-glucosidase inhibition. Still on the start zone, a strong cholinesterase inhibitor is evident (zone d).

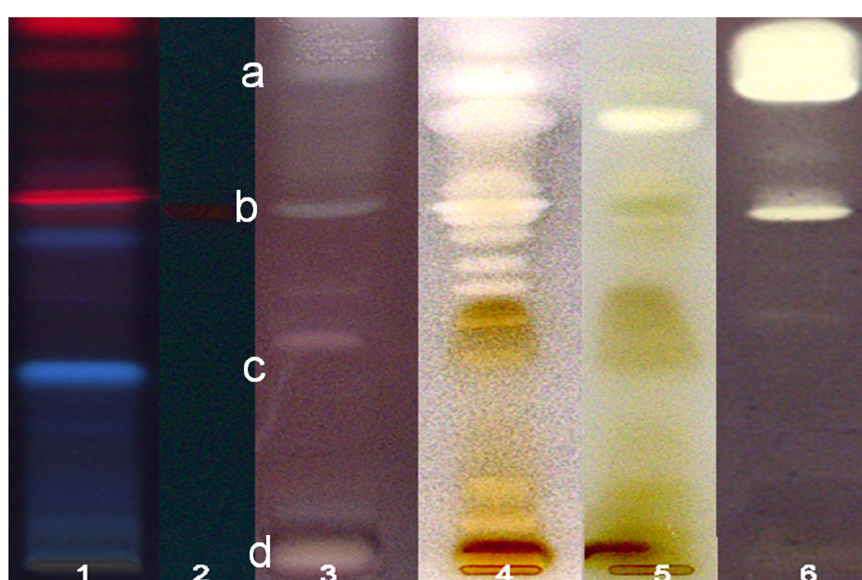

Fig. 3 Chromatogram of *S. officinalis* (1) and luteolin solution (2) with natively fluorescent zones at UV 366 nm detected with cholinesterase (3), α- (4) and β-glucosidase (5) and *Bacillus subtilis* (6) bioassay.

HPTLC-Bacillus subtilis

The most active antimicrobial substances were in the nonpolar chromatographic hR_F range. Substance zone a generated a strong antimicrobial effect by HPTLC-*Bacillus subtillis* (Fig. 4, A). In the nonpolar separation (Fig. 4, B), substance zone b seemed to be separated into two zones assigned as b', applied approximately half in volume.

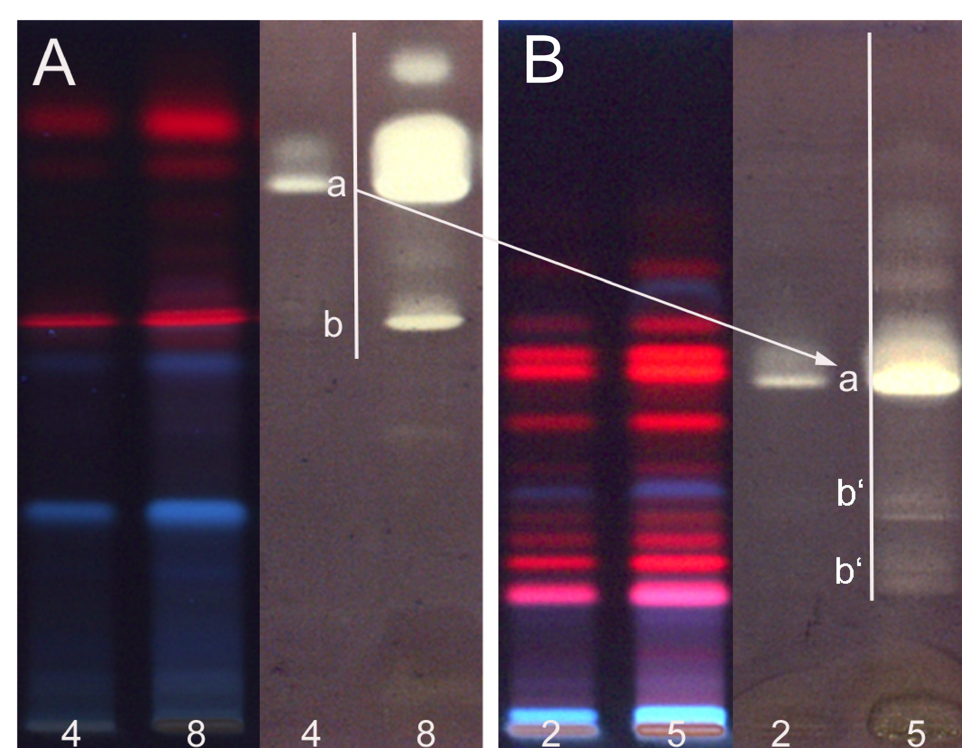

Fig. 4 Chromatogram of *S. officinalis* with natively fluorescent zones at UV 366 nm and after *Bacillus subtilis* bioassay at white light illumination showing antibiotics (polar (A) and apolar (B) separation; numbers: µL-volumes applied).

The HPTLC densitograms of *B. subtilis* demonstrated that it is possible to quantify the antimicrobial substances in reference to a standard after identification (Fig. 5). For latter, high-resolution MS and NMR can be employed directly from the HPTLC plate after elution via the TLC-MS Interface.

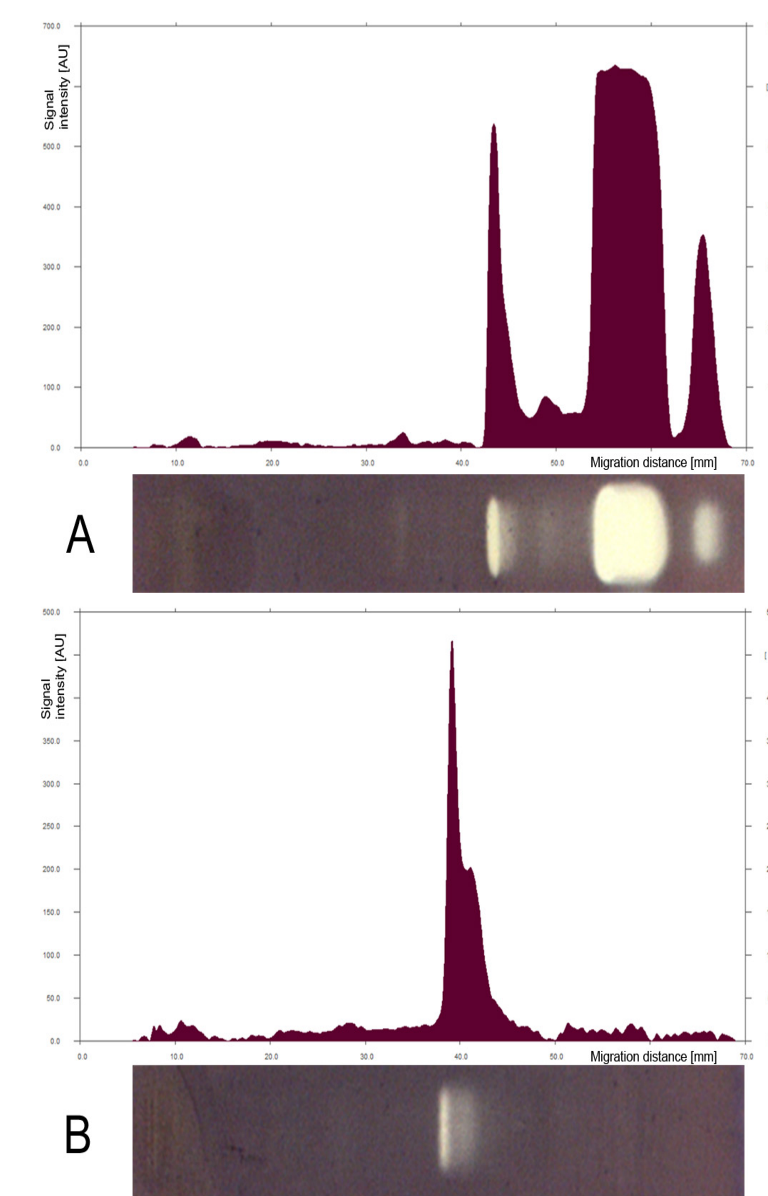

Fig. 5 Densitometry (absorbance measurement at 546 nm using mercury lamp) of the *B. subtilis* bioautogram (opposite measurement mode (fluorescence) to circumvent the automatic software inversion of the measured absorbance signal).

HPTLC-ESI-MS

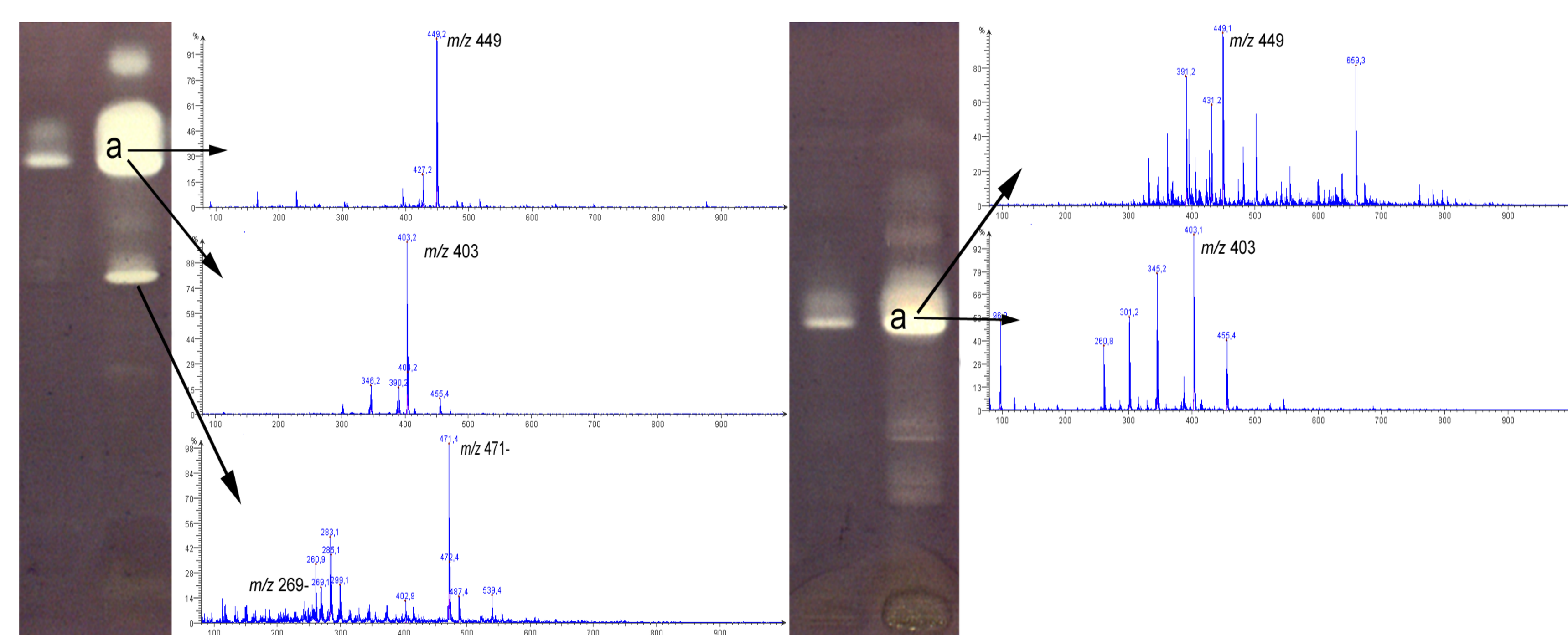

Fig. 6 HPTLC-ESI-MS of the antimicrobial substances a and b in *S. officinalis* (A) after polar and (B) apolar separation

Zones showing an antimicrobial effect were eluted from the HPTLC plate via the TLC-MS Interface directly into the ESI-MS (Fig. 6). For reduction of the plate and system background, a blank mass spectrum was subtracted from the analyte mass spectrum. The HPTLC-MS full scan mass spectrum of zone a showed clear basepeaks at *m/z* 449 (ESI⁺) and *m/z* 403 (ESI⁻) for both separations. For zone b, a basepeaks at *m/z* 471 (ESI⁻) was obtained.

Single bioactive compounds were detected in *S. officinalis* using effect-directed analysis, which are further characterized by high-resolution MS.

References [1] J. Yang, L. Choi., D. Li, F. Yang, L. Zeng, J. Zhao, S.P. Li, J Planar Chromatogr 24 (2011) 257–263, [2] C.A. Simões-Pires, B. Hmicha, A. Marston, K. Hostettmann, Phytochem Anal 20 (2009) 511–515.

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