Potential applicability of modern bioautography (BioArena) in the study of plant ingredients

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TLC- and OPLC-bioautography

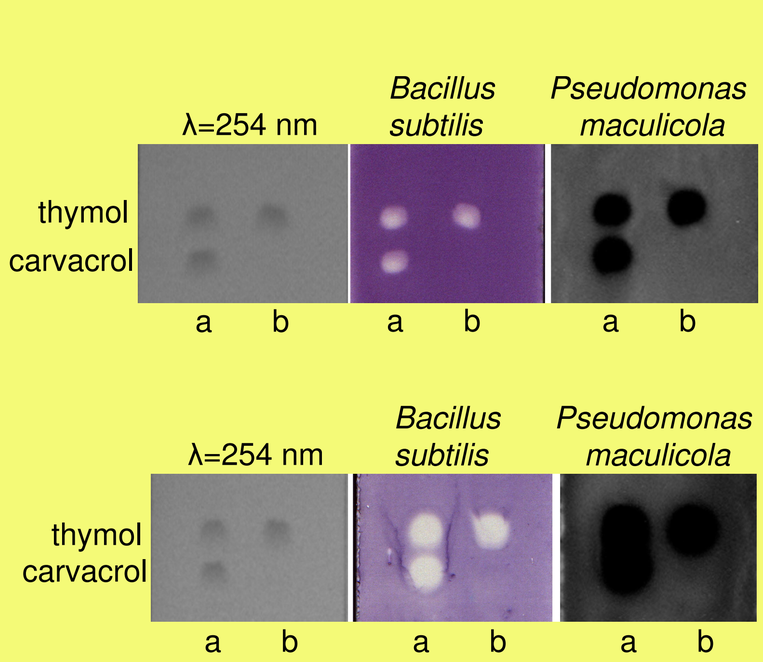
Direct bioautography, the combined application of planar layer liquid chromatographic separation (TLC, HPTLC, OPLC) and post-chromatography bioassay, enables the detection of antimicrobial components e.g. of plant extracts. In the course of the biological detection the adsorbent layer after development is dipped into or sprayed with given cell suspension and afterwards the bioautogram is visualized.

Visualization of the bioautogram is usually performed **by** the use of **tetrazolium salts** (see bioautograms using ***B. subtilis***). The living cells reduce the yellow tetrazolium salts to bluish formazan, so the antimicrobial active compounds appear as clear spots/zones against a darker background.

Using the **emitted light as signal**, the performance of biodetection is very easy. The image of the bioautogram (see the use of **luminescent gene tagged *P. maculicola***) can be directly recorded by a cooled camera in a dark box.

Because of the overloaded condition there is no characteristic differences in the separation efficiency comparing the use of TLC and HPTLC adsorbent layer. However the biological detection is more sensitive in the case of HPTLC layer, which may be the result of the differences between TLC and HPTLC in thickness, binding material, pH and/or the concentration of trace elements.

The influence of the quality of the adsorbent layer on the biological detection

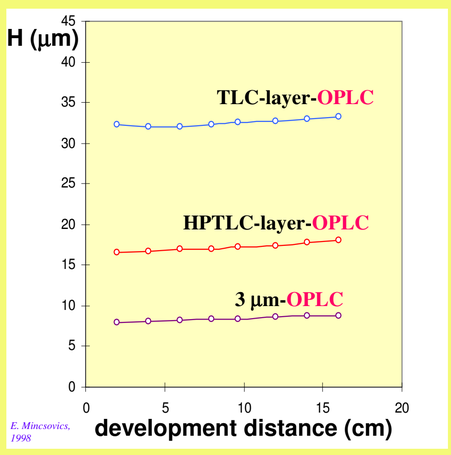


Infusion OPLC, 20x20 cm TLC/HPTLC layer, (dried 130 ^{0}C/3 h), 5MPa, 300/450 µL/min, chloroform, total volume 5540/4250 µL, 918/572 s.
a - standards, 3 µg of each thymol and carvacrol
b - Thymus essential oil (16 µg)

Conventional TLC/HPTLC chromatography with chloroform-acetone 9:1 (v/v).

Overpressured Layer Chromatography (OPLC)

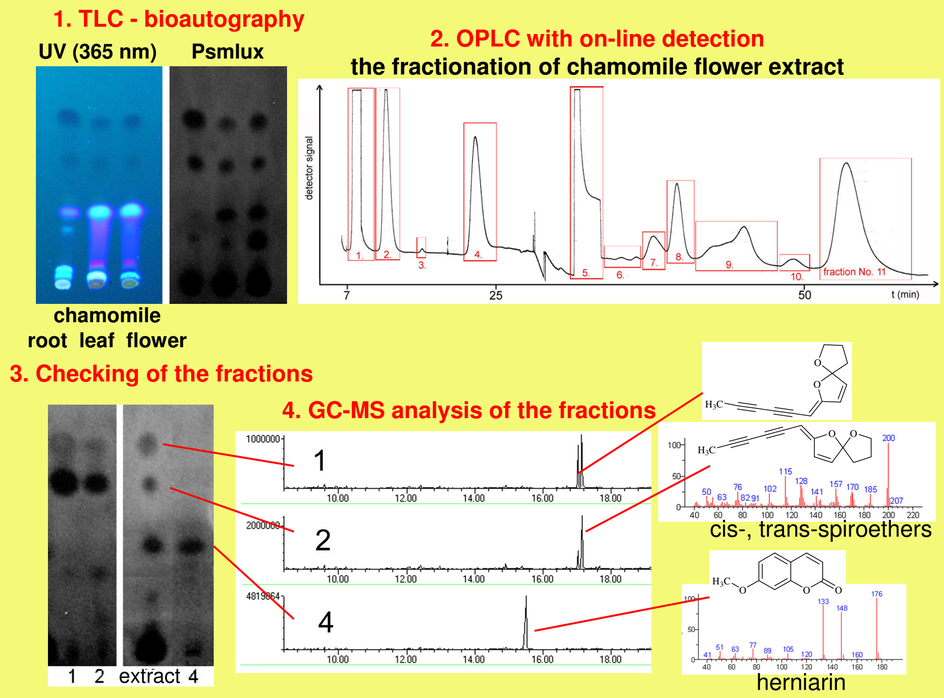
OPLC 50 system



In OPLC system the mobile phase migrates through the entire layer, being under homogeneous pressure (50 bar), with constant velocity that is achieved by the application of a pump system. The forced flow leads to a faster separation and makes possible the longer development distance, increasing zone capacity. Constant velocity results in almost constant average theoretical plate height (H) in the whole development distance.

The OPLC system has a high flexibility. The principal operation steps as sample application, separation, detection and isolation can be freely combined. The fully off-line process (spotting/streaking sample application & separation & *in situ* densitometric evaluation) started with dry non-segmented adsorbent layer corresponds to TLC. The fully on-line OPLC (injection & flow-cell detection) performed on a conditioned adsorbent layer is analogous with HPLC and at the same time single sample can be processed. Isolation of important components can be carried out off-line by elution from the chromatographic spots/bands scraped off or on-line by collecting their peaks after flow-cell detection using an overrun.

Identification of antibacterial components of plant extract using OPLC with on-line detection and GC-MS

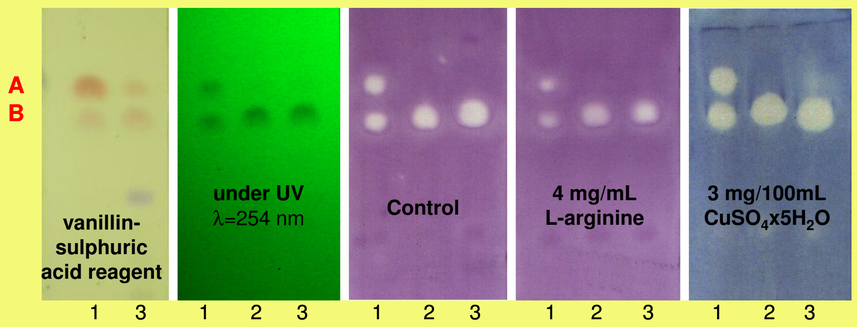


BioArena investigations

The chromatographic BioArena means the coordination of the operating steps in biological detection of potential ingredients as well as the unlimited use of biochemicals/chemicals for interactions with the cells in chromatographic spots/bands. BioArena, beyond the detection of antimicrobial components, is also appropriate for examination of the mechanism of cell proliferation inhibition and/or promotion effects. The influence of different endogenous and/or exogenous substances on the bioactivity of separated compounds can be examined by dissolving substances in the cell suspension just before inoculation. After biological detection there is a possibility of in situ quantitative densitometric evaluation in addition to in situ and ex situ qualitative and quantitative investigations, for example other chromatographic separations, IR, FT-IR, FT-Raman, NMR spectroscopy, LC/GC–MS, and MALDI-MS.

The antibacterial activity of *O. onites* components and aflatoxins (AFs) was investigated against *Bacillus subtilis* in BioArena. In the presence of formaldehyde (HCHO) capturer (e.g. L-arginine) their antimicrobial activity (the inhibition zones) was decreased comparing with the control layer. If HCHO generator and transporter Cu(II) ions or HCHO precursor N^G-monomethyl-L-arginine (MMA) were dissolved in the culture medium the antibacterial effect were increased characteristically (not shown all results). The FT-Raman spectra, obtained *in situ* around the AFB1-containing spots in bacterium-free and inoculated TLC layers, indicates an excess of HCHO formation by demethylation of AFB1 at its methoxy group in the presence of bacterial cells (the intensity of the δCH_3 band of AFB1 (1386 cm^{-1}) was reduced by 50%). It seems that these compounds generate antimicrobial activity through HCHO and its reaction products.

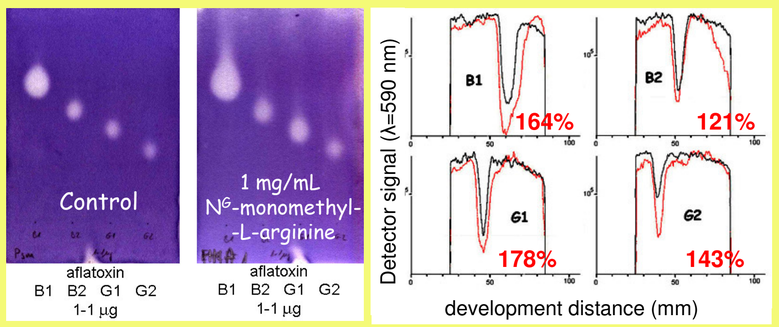
The effect of L-arginine and Cu(II) ions on the antibacterial effect of *Origanum onites* oil components



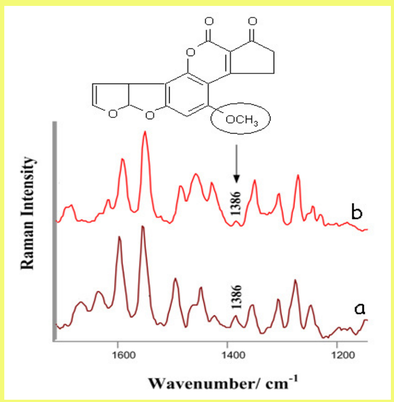
1. Standards, 10 µg of each thymol (A) and carvacrol (B);
2-3. Origanum oils (30 µg of each)

Infusion OPLC, 20x20 cm TLC layer, (dried 130 ^{o}C/3 h), 5MPa, 400 µL/min, dichloromethane, total volume 4847 µL, 738 s.

The influence of MMA on the antibacterial effect of aflatoxins and its densitometric confirmation



„Off-line" OPLC, 20x20 cm TLC layer, (dried 130 ^{o}C, 3 h), 5MPa, 400 µL/min, chloroform-acetone 9:1 (v/v), total volume 4500 µL, 685 s.



Surface enhanced FT-Raman spectra of (a) the aflatoxin B1 spot in bacteria free TLC layer and (b) the aflatoxin B1 spot in TLC layer inoculated with Psm cell suspension

OPLC (Overpressured layer chromatography) separations were performed by Personal OPLC BS50 system (OPLC-NIT, Budapest, Hungary).
Method of Bioassay: The dried chromatoplates were dipped into the soil bacteria *Bacillus subtilis* cell suspension (10 s). After 1 hour incubation the antibacterial assay was visualized with aqueous solution of dye reagent MTT (Sigma Aldrich Ltd., Budapest) (dipping into for 5 s). In BioArena, studying the influence of L-arginine (Sigma) or Cu(II) (Reanal, Budapest) on the antibacterial activity of oil components we dissolved them in the bacterial cell suspension before inoculation.
For luminescent detection the adsorbent layers dipped into luminescence gene tagged *Arabidopsis* pathogen *Pseudomonas maculicola* cell suspension and were placed in a glass cage keeping them wet in a closed air atmosphere. Bioautograms were acquired under a computer-controlled cooled CCD camera (IS-4000; Alpha Innotech, San Leandro, USA) and documented immediately after the inoculation.

References
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