Standardized bioautographic methods for effect directed screening of samples separated on HPTLC plates

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Summary

HPTLC is a convenient technique in the search for new bioactive compounds from natural products or other sources. Biological and chemical screening can easily be performed on the plate after separation of a complex matrix.

This poster presents three bioautographic assays based on totally different approaches. The first one is an enzymatic assay. After development, spraying α- or β-glucosidase on the plate and a short incubation time the viable enzymes are detected with an adequate substrate and a color reaction. The second assay is performed with bioluminescent bacteria. Their inhibition is visible as bioluminescence quenching zones. The third assay is based on a physico-chemial detection with stable, purple DPPH$^{\bullet}$ reagent. All three approaches are easy to handle and can be performed in a conventional laboratory. They are rapid, economical and efficient screening assays.

Introduction

Effect directed bioautographic screening by HPTLC is an important tool in the search for new bioactive compounds from medicinal plants and other sources. It is also an additional detection mode for unknown constituents of samples. The advantage of in-situ bioautographic methods in HPTLC results from the hyphenation of a separation of complex samples with subsequent biological tests. A simplified sample preparation and instant results are additional benefits of the techniques.

We adapted three bioautographic methods for in-situ detection and combined them with the high reproducibility and separation power of state of the art HPTLC. The different types of bio-assays can be performed directly on the plate without or with just a short incubation time. Several method parameters had to be standardized, particularly the immersion or spraying procedure, as well as the exposure and incubation time. Three bioautographic assays were studied:

Enzymatic assay:
Inhibition of α- and β-glucosidase

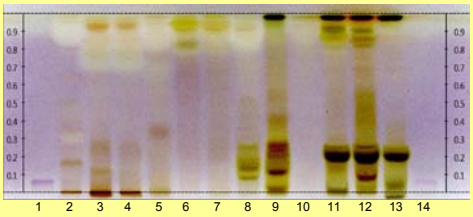

Fig. 1 α-glucosidase: Screening of (track) 1/14: acarbose, 2: Ziziphus, 3/4: Acorum, 5: Maitake, 6/7: Piper, 8: Acantho-panax, 9/10: Urtica, 11: Salvia, 12: Thymus, 13: Melissa; MP: Chloroform, methanol, water = 70:30:4

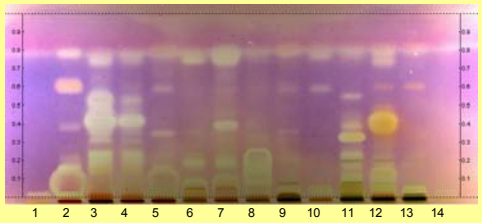

Fig. 2 β-glucosidase: Screening of different plant species (as in Fig. 1), MP: Toluene, ethyl acetate = 95:5

Cellular detection process:
Inhibition of bioluminescent bacteria
Vibrio fischeri

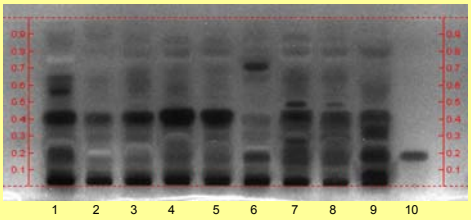

Fig. 3 Screening of different Zanthoxylum species, MP: n-Hexane, ethyl acetate = 16:5

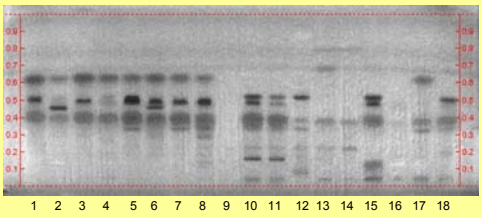

Fig. 4 Screening of water miscible cooling lubricants (coolant) for bacteriostatic acitvity.
We thank Dr. R. Weber (Blaser Swisslube AG) for the contribution of this image.

Physico-chemical detection:
Reduction of DPPH$^{\bullet}$ radical to DPPH

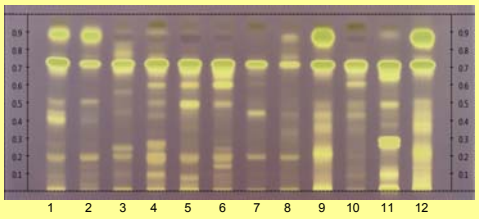

Fig. 5 Screening of flavonoid containing drugs from the Lamiaceae family for antioxidant properties, MP: Ethyl acetate, formic acid, water = 15:1:1

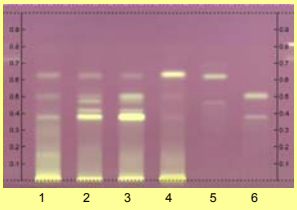

Fig. 6 Antioxidant activity of black tea (track 1) and green tea extract (track 2-4), track 5/6: different catechins, MP: Toluene, acetone, formic acid = 9:9:2

Material

ADC2 with humidity control, immersion and spraying device, ATS4, Visualizer (all CAMAG), HPTLC Si 60 F254 (Merck); p.a. solvents (Acros, Merck, Roth, Sigma-Aldrich, Carbosynth, ChromaDex); standards (Bayer, Extrasynthese, Sigma-Aldrich), and plant samples from different origins.

Method

1g of powdered plant material is mixed with 10mL of methanol and sonicated for 15min, then centrifuged or filtered (for extract: 20-400 mg in ethanol/water 80:20). 150mg of acarbose is dissolved in 5mL of methanol (Fig. 1 and 2), 1mg of bergapten in 1mL of methanol (Fig. 3), 1mg of catechins in 5mL of methanol (Fig. 6). Spray-on application of 1-10µL as 8mm bands. Development in ADC2, 20min chamber saturation, developing distance 70mm, mobile phase MP (see Figures), plates conditioned to 33% RH. Derivatization by spraying the adequate substrate together with Fast Blue B Salt (Fig. 1 and 2, [1]) or immersion into *Vibrio fischeri* suspension (Fig. 3 and 4, [4]) or immersion into 0.05% DPPH$^{\bullet}$ in methanol (validated method [5]).

Literature

[1] C. A. Simões-Pires et al., Phyto-chem. Anal. 20, **2009**, 511-515.
[2] W. Weber et al. CAMAG CBS 94, **2005**, 2-4.
[3] K. Hostettmann et al., J. Planar. Chrom. 10, **1997**, 251-257.
[4] www.bioluminex.com
[5] CAMAG Application Note F-38: http://www.camag.com/laboratory/methods/identification.html

Results and discussion

The inhibition of α- and β-glucosidase is an important target in the search for pharmaceutical ingredients active against type 2 diabetes and anti-viral infections [1]. The positive response is visible as white zones on the HPTLC plate (Fig. 1 and 2). The screening test is able to detect specific or unspecific inhibition of α- and β-glucosidase with positive control or compounds previously separated from a complex mixture. The advantage of this enzyme assay is the short incubation time of 1 h and the easy handling in a conventional laboratory.

The bioluminescent bacteria *Vibrio fischeri* used for activity-related detection reveal toxicological properties of plant species and pure substances. The dark zones on the HPTLC plate indicate bioactive compounds. Beside the detection of potential toxicity the assay is effective for obtaining a fingerprint which can help to identify adulteration of samples (Fig. 3 and 4). This bioassay can be used as research tool to identify biologically active constituents of complex mixtures - among others natural products, drinking water and wastewater [2].

The radical scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) is suitable for detecting antioxidant properties of substances from medicinal plants or pure compounds [3]. Rosmarinic acid (Rf = 0.73) demonstrates significant free radical scavenging activity together with other flavonoids (Fig. 5) and phenolic compounds from green and black tea (Fig. 6). The screening for antioxidative compounds is also of interest to manufacturers of new cosmetic formulations.