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HYPHENATION OF HPTLC WITH BIOAUTOGRAPHY AND MALDI-TOF-MS AS AN EFFECTIVE TOOL FOR BIOPROCESS AND QUALITY CONTROL OF PLANT EXTRACTS

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Bioassays are usually performed in water based buffers or additionally substrate containing broths. Solvents are usually disturbing in these assays. By direct performance of visual detectable enzyme reactions on the HPTLC plate, the separated natural compound library can also be used for activity screening if a visualization of the assay is feasible.

Enzymatic bioautographic assays using Xanthine Oxidase (XOD)¹, Lipase, Acetylcholinesterase (AChE)² and β -Glucosidase³ were optimized and applied for screening of less studied medicinal plants extracts and fractions from conventional and in vitro cultivation. HPTLC standardized fingerprint analysis was performed with automated equipment from (CAMAG, Muttenz) and HPTLC plates (Merck, Darmstadt).

It was observed that this on first sight very simple technique could deliver false positive results. Since a visualisation reaction is required, several conditions can lead to seeming inhibition spots. In truth, lipophilic compounds could prevent wettability of the silica, which hindered the enzyme reaction at this spot. Moreover, reaction of assay compounds with visualization reagents could also cause visible artefacts. Results of screening of in vitro medicinal plant cultures and detection of artefacts are presented in detail. Extracts of in vitro cultured *Sideritis scardica* and *Pulsatilla slaviankae* showed to contain active compounds that are able to inhibit AChE and β -glucosidase. AChE inhibitors were moreover identified in *Clinopodium vulgare* whereas *Pulsatilla montana* showed potential XOD inhibiting compounds.

Direct coupling of HPTLC with MS detectors, e.g. MALDI-TOF-MS might be a suitable tool for detection of the active spots. For the analysis of flavonol aglycones and glycosides it has recently been reported to be applicable by Krosiakova and Wolfram⁴. The HPTLC chromatograms can be scanned with this technique for MS data from the start to the front zone.

In conclusion, bioautographic enzyme assays and HPTLC-MS hyphenation offer a rapid and simple tool for screening of secondary metabolite profiles for potential health beneficial activities, indeed active spots need to be examined critically.

References: ¹Ramallo (2012). *J Med Chem* 8(1):112-117, ²Hassan (2011). *Phytochem Anal*: 23(4): 405-407. ³Marston et al. (2002). *Phytochem Anal*: 13(1): 51-54. ⁴Krosiakova and Wolfram (2016). *Phytochem Anal*: 13(1): 51-54.

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