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IL6: Enzymatic bioautography on HPTLC: combined phytochemical and activity screening tool for medicinal plants quality assessment and *in vitro* cultivation bioprocess control

Sarah Bräm¹, Beat Meier¹, Kalina Danova², Evelyn Wolfram^{1,*}

¹Zurich University of Applied Sciences (ZHAW), Institute of Biotechnology, Phytopharmacy Research Group, Wädenswil, Switzerland

²Institute of Organic Chemistry Centre of Phytochemistry, Bulgarian Academy of Science, Sofia, Bulgaria

ABSTRACT

High Performance Thin Layer Chromatography (HPTLC) is an analytical tool of long term tradition in quality control of medicinal plant extracts and other natural multi compound mixtures. It is a rapid and standardizable technique, which delivers an informative picture of separated compounds as a result, which are fixed on the solid silica phase of the plate – like a compound library. By direct performance of visualizable enzyme reactions on the plate, this compound library can also be used for activity screening, called enzymatic bioautography. Similarities and differences of phytochemical and activity fingerprints can be compared for quality control purposes as well as for bioprocess control for *in vitro* medicinal plant cultivation. Objective: The application of direct coupling of enzymatic assays and phytochemical fingerprint analysis by HPTLC shall be demonstrated. Assays procedures are optimized and applied for screening of medicinal plants extracts and fractions from conventional and *in vitro* cultivation.

Methods: HPTLC standardized fingerprint analysis by automated equipment (CAMAG, Muttenz) and HPTLC plates (Merck, Darmstadt). Derivatization by Neu's and PEG reagent. Detection at different wavelength. Optimization of published methods: Xanthine Oxidase (XOD) assay by Ramallo 2011; Lipase assay by Hassan 2011. Acetylcholinesterase (AChE) assay Marston et al 2002.

Results: When attempts to reproduce bioautographic enzyme assays from literature failed, extended optimization experiments resulted in validated, robust procedures. Screening of less studied medicinal plant species from the Balkan region from conventionally grown and *in vitro* cultivated origin showed the following results:

	XOD	Lipase	AchE
<i>Hypericum sp.</i>	+ (1-2)	-	(+)
<i>Pulsatilla sp.</i>	+ (2-4)	+ (1)	(+)
<i>Inula britannica</i>	+ (1-2)	(+)	-
<i>Sideritis scardica</i> .	-	+ (1)	+ (2-4)
<i>Artemisia alba</i>	+ (1)	-	-
<i>Clinopodium vulgare</i>	+ (2-3)	++ (1)	+(1)

Legend: + inhibition, (+) faint inhibition, – no visual inhibition, (number) number of inhibition zones

Conclusion: Bioautography offers a rapid and simple tool for screening of secondary metabolite profiles of medicinal plant biomass and derived products by HPTLC combined with screening of potential health beneficial activities. Direct coupling with MS detection (HPTLC-MS Interface or HPTLC-MALDI-TOF) could yield rapid additional mass information of the active molecule.

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