

Year

2015

Issue

18: 1645-1743 | E6

17: 1569-1643 | E5

16: GA 2015 – Book of Abstracts

15: 1309-1391 | E4

14: 1223-1307 | E3

12/13: Special Issue Prof. Dr. J. H. W. Lam

11: American Society of Pharmacology and Therapeutics

10: 771-854

> Table of Contents

> Current Issue

> Sample Issue (01/2019)

Medizin und Markt

> Produkt- und Therapie-Informationen der Industrie

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> Pharmacopsychiatry

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Enzymatic bioautography on HPTLC: combined phytochemical and activity screening tool for quality assessment and in vitro cultivation bioprocess control for selected medicinal plants from the Balkan region

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Congress Abstract

HPTLC is a tool of long term tradition in medicinal plants analysis. Separated compounds are fixed on the solid silica phase like a compound library. By direct performance of visualizable health relevant enzyme reactions on the dried plate, similarities and differences in the phytochemical and activity fingerprints can be identified.

In this work, enzymatic bioautographic inhibition assays, using xanthine oxidase (XOD) [1]; lipase [2] and acetylcholinesterase (AChE) [3] have been optimized and applied for screening to show challenges and pitfalls.

Merck HPTLC plates were used on CAMAG equipment. Flavonoid fingerprints, derivatized with Neu's/PEG reagent were detected at 366nm. Validated, robust procedures have been established. False positive results are avoided by detection at different wavelength.

Fingerprints of less studied, *in vitro* cultivated medicinal plant species (see Table 1: Detection and number of enzyme inhibition zones for the *in vitro* cultivated species from the Balkan region using bioautography) from the Balkan region showed the following enzyme inhibitory results:

	XOD	Lipase	AChE
<i>Hypericum</i> sp.	+ (1 – 2)	-	(+)
<i>Pulsatilla</i> sp.	+ (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

A binary answer for the activity of the separated zones appears visually on the HPTLC chromatogram. Application of positive controls on the silica layer as a concentration ladder allows for intensity benchmarking as a qualitative control activity equivalent.

It can be concluded, that bioautography offers a rapid and simple tool for screening of secondary metabolite profiles combined with enzymatic inhibition screening by HPTLC, either for quality control purposes or bioprocess control for *in vitro* cultivated medicinal plant biomass. Such assays can complement sophisticated assays to reduce the number of samples.

Acknowledgements: BSRP, grant No. IZEBZ0, 142989; DO2 – 1153

[1] Ramallo IA et al. *Phytochemical Analysis* 2006; 17: 15 – 19.

[2] Hassan AMS, *Phytochemical Analysis* 2011; 23: 405 – 407.

[3] Marston A et al., *Phytochemical Analysis* 2002; 13: 51 – 54.



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Relations between polyphenolics production and enzymatic antioxidant defense in *Pulsatilla montana* ssp. *balcana* in vitro

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Congress Abstract

Asian *Pulsatilla* species are utilized in traditional medicine as anti-inflammatory, spasmolytic, anti-enteritis and antitumor remedies. Little information is still available on the chemical composition and properties of the Balkan representatives of the genus.

Column and thin layer chromatographic separation of the methanolic extract of the aerals of Balkan endemic *P. montana* ssp. *balcana* led to isolation of miquellianin, caffeic and 3,5-dicaffeoylquinic acids as main components, which were determined by spectroscopic methods. In addition, tiliroside, hyperoside and isoquercitrin were confirmed by thin layer chromatography with authentic samples. Further on, shoot cultures of the plant were developed and treated with plant growth regulators in order to modify developmental patterns and study the enzymatic and non-enzymatic antioxidant defense of the plant *in vitro*. Indole-3-butyric acid stimulated the antioxidant enzymes phenylalanine ammonia-lyase, superoxide dismutase, catalase and glutathione peroxidase, but still increased oxidative stress (determined by the levels of hydrogen peroxide *in vitro*) and intensive callusogenesis were observed, worsening the quality of obtained explants. HPTLC screening showed reduced polyphenolics and DPPH scavenging capacity of these samples. On the contrary, 1-naphthaleneacetic acid led to inhibition of the activity of these enzymes, but stimulation of glutathione reductase and ascorbate peroxidase, as well as elevated non-enzymatic antioxidants as ascorbate and polyphenolics were observed, related to formation of normal rosette clumps and improved radical scavenging capacity of the plants. The results are indicative of the possible interrelations between enzymatic and non-enzymatic antioxidant defense in the plant which might be used as a tool for the optimization of polyphenolics production *in vitro*.

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- 14: 1223-1307 | E3
- 12/13: Special Issue Prof. Dr. G. W. Knebel
- 11: American Society of Pharmacognosy
- 10: 771-854

> Table of Contents
> Current Issue
> Sample Issue (01/2019)

Medizin und Markt

> Produkt- und Therapie-
Informationen der
Industrie

Related Journals

> Planta Medica
International Open
> Drug Research

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Optimization of an UHPLC method for flavonoids from Hypericum species

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Congress Abstract

The broad spectrum of pharmacological activities of Herba Hyperici preparations is determined by the potential additive effect, synergism or even possible antagonism of this multicomponent system.

The *in silico* assisted development of an analytical chromatographic method for acquisition of a well separated fingerprint of flavonoids, suitable for bioprocess control of conventionally and biotechnologically derived plant material of *Hypericum* species is presented here. Drylab[®] software is based on modelling of physicochemical phenomena in an LC system and supports efficient method development by experimental design models. *H. perforatum*, as well as species with indigenously high (*H. richeri*, *H. rumeliacum*) and lacking hypericin production (*H. calycinum*) were selected. Separation was performed on ACQUITY UPLC (Waters) system with PDA Detector. DryLab[®] software (Molnár Institute) was used to model and predict experiments for the optimization of UHPLC conditions establishing an appropriate method for separation of flavonoid compounds. The fingerprint and the identification of the well described lead flavonoids rutin and hyperoside for the quantification of flavonoids as a sum parameter are suitable for comparison of *Hypericum* samples from different accessions or bioprocess conditions. It was confirmed that the predicted chromatogram matched the peak order in the fingerprint analysis of a real sample from *H. calycinum* extract. The analysis of the samples with the *in silico* optimized method revealed that *ex situ* sample of *H. perforatum* has content of around 9 – 11 µg/100µg of total flavonoids calculated as hyperoside of all species tested. Among the different *in vitro* samples, the flavonoid content varied in the order of magnitude of 2 – 7 µg/100µg. It was demonstrated that the *in silico* assisted optimized UHPLC method is suitable for bioprocess control of flavonoids in *in vitro* and *ex situ* biomass.

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