

Improved Bioautographic Xanthine Oxidase Assay: Combining HPTLC separation and activity assessment for rapid and cost effective activity detection in medicinal plant biomass

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Bioautography offers a rapid and simple tool for discovery of active health beneficial secondary metabolites of medicinal plants by HPTLC [1]. Xanthine Oxidase (XO) catalyzes the oxidation of hypoxanthine and xanthine to uric acid under the formation of superoxide radicals and hydrogen peroxide. An overproduction of these reaction products in the human body is associated with diseases such as hyperuricemia, gout, hypertension, diabetes and different inflammatory diseases.

The aim of this work has been to optimize and validate a bioautographic XO inhibition assay first described by Ramallo et al. (2006) to obtain reliable and reproducible results for screening of medicinal plant biomass of conventional or biotechnological origin.

The assay procedure has been improved by optimizing XO activity, buffer conditions as well as the concentrations of redox dye and substrate. XO inhibitory effects were visualised as white zones on a purple coloured thin layer chromatogram based on the reaction of superoxide radicals with nitroblue tetrazolium chloride.

The visual detection limit of the competitive XO inhibitor allopurinol was 45.4 ng. An extract of green tea (*Camellia sinensis*) as a known natural XO inhibitor could be visually detected down to an applied amount of 10 µg extractives dry weight (dw).

Screening of different in vitro cultured *Hypericum* species from the Balkan region elucidated three very distinct XO inhibiting zones in *Hypericum calycinum* two of them comparable in intensity to 2.5-5 µg Allopurinol as positive control. Some less pronounced zones (ca. ≤1 µg Allopurinol) could be detected in *H. richeri*, *H. rumeliacum* and *H. perforatum*.

From the results it can be concluded, that the improved bioautographic XO inhibition assay is a rapid and valid research tool for assessment of active secondary metabolites from in vitro cultured or conventionally grown medicinal plants for actives discovery or bioprocess control during cultivation studies.

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References:

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2. Ramallo I.A., Zacchino S.A., Furlan R.L.E. (2006). *Phytochemical Analysis*. 17: 15-19.1.