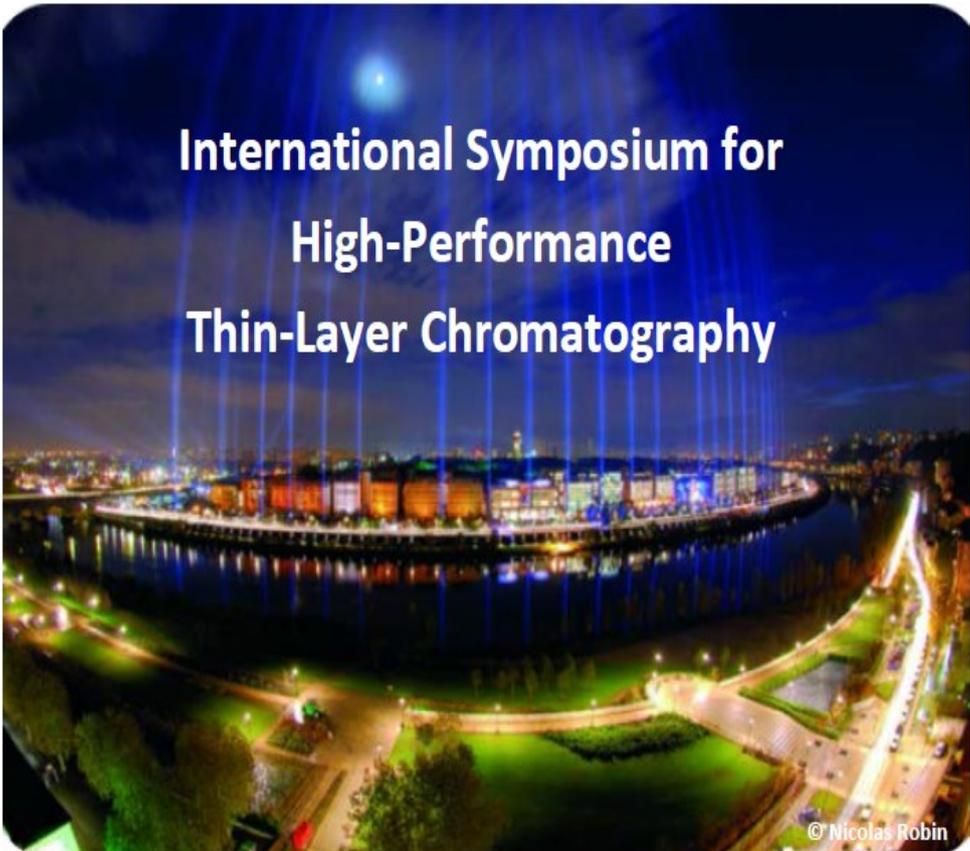


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Improved bioautographic xanthine oxidase assay: combining HPTLC separation and activity assessment for phytopharmaceutical research

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Bioautography offers a rapid and simple tool for screening of secondary metabolite profiles of medicinal plants by HPTLC combined with screening of potential health beneficial activities. The aim of this work has been to optimize and validate a bioautographic Xanthine oxidase (XO) Inhibition assay first described by Ramallo *et al.* (2006) to obtain reliable and reproducible results. Xanthine oxidase (XO) catalyses the oxidation of hypoxanthine and xanthine to uric acid under the formation of superoxide radicals and hydrogen peroxide. XO inhibition is a valuable assay in bioactivity screening for active ingredient discovery.

The assay procedure has been improved by optimizing concentrations of redox dye, substrate, enzyme activity as well as the buffer conditions in combination with low gelling agarose and adjustment of incubation time and temperatures according to the XO thermal activity characteristics. 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. Extracts of *Camellia sinensis* and *Artemisia alba* showed also to contain constituents with XO inhibitory activity, that could be visually detected down to an applied amount of 10 µg dry weight (dw) for *C. sinensis* extract and 100 µg dw for *A. alba*.

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 medicinal plants.

[1] Ramallo I.A., Zacchino S.A., Furlan R.L.E. (2006). *Phytochemical Analysis* 17: 15-19.

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