Bioactivity profiling of *Salicaceae* bud extracts

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Introduction

Salicaceae Mirbel is a family of shrubs and trees present in all the regions of the world. The most known species, which were abundantly used in traditional medicine, belong to two genera of the *Saliceae* Rchb. tribe: *Populus* L. (poplars) and *Salix* L. (willows) [1]. The buds of these trees are increasingly used in gemmotherapy; moreover, they importantly contribute to the production of propolis by European bee *Apis mellifera* L. [2]. An HPTLC method hyphenated to several bioassays was developed, with the purpose to detect antibacterial and phytoestrogenic chemical constituents, as well as inhibitors of acetylcholinesterase, respectively.

Results and discussion

Extract preparation, separation and derivatization

Twenty-one *Salicaceae* bud samples were collected in Serbia, from *Populus nigra* L. (1-11;14-21), from *Populus alba* L. (12) and from *Salix herbacea* L. (13). They were extracted with 30 mL ethanol 80 % in an ultrasonic bath, filtrated and dried. The separation was carried out on HPTLC plates silica gel 60, with *n*-hexane, ethyl acetate and acetic acid, under fuming HCl atmosphere [3]. The polyphenols were derivatized with Neu's reagent and PEG 400, showing five different general patterns at UV 254 nm and UV 366 nm (Fig.1 A and B). For comprehensive information on the bioactivity profile of these 21 samples, various bioassays were employed next.

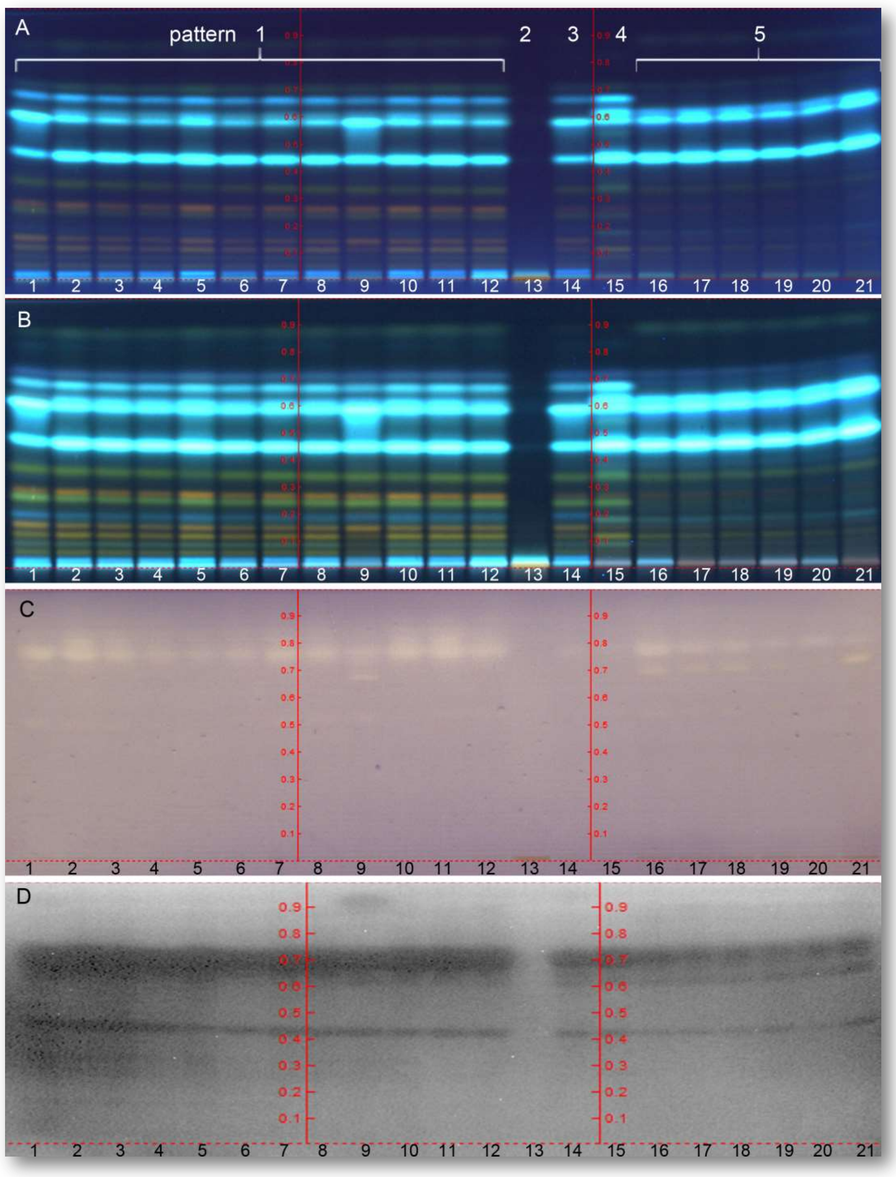

Fig. 1 Chromatograms of *Salicaceae* bud extract samples sorted according to the five patterns (A) after derivatization with natural product reagent at UV 254 and (B) at UV 366 nm as well as after application of the (C) *Bacillus subtilis* and (D) *Aliivibrio fischeri* bioassays revealing several bioactive compounds.

Bioassays

First, effect-directed analysis (EDA) of antimicrobial and microbioactive compounds was done with *Bacillus subtilis* (Fig. 1 C) and *Aliivibrio fischeri* (Fig. 1 D, according to *DIN EN ISO 11348-1*), respectively. At least two unpolar compounds found in *Populus* samples have antimicrobial activity against *B. subtilis*, whereas 3 compounds present in all *Populus* samples were detected as active in the *A. fischeri*.

Secondly, we submitted the samples to the modified protocol of the planar yeast estrogen screen (pYES) for detection of substances with phytoestrogenic activity (Fig. 2 A). One phytoestrogenic compound was found in all *Populus* samples (Fig. 2 B).

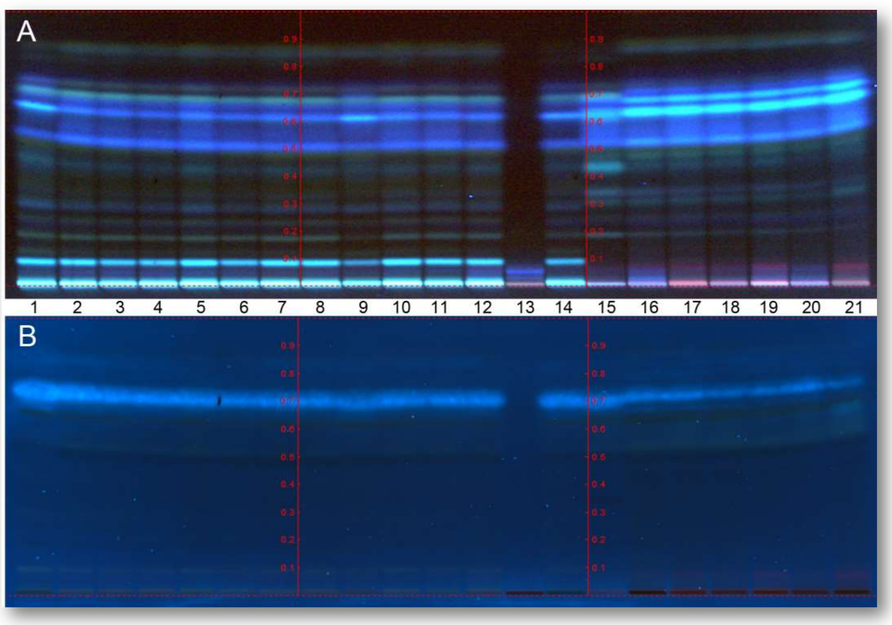

Fig. 2 Chromatograms of *Salicaceae* bud extract samples at UV 366 nm after (A) chromatography and (B) pYES bioassay.

Thirdly, the optimized [4] Marston's colorimetric method [5] was adapted and applied for the identification of acetylcholinesterase inhibitors (Fig. 3). At least two inhibitors were found in *Salix*, but far more in *Populus* buds.

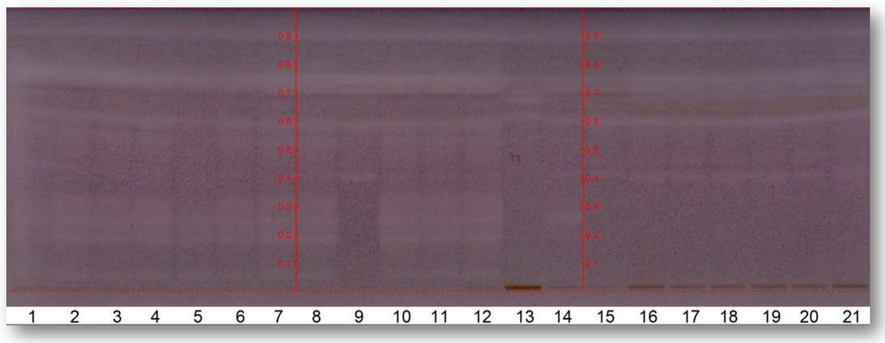

Fig. 3 Chromatograms of *Salicaceae* bud extract samples at white light illumination after AChE enzymatic assay.

Conclusions

HPTLC hyphenation (with a parallel analysis of up to 21 extract samples on one plate) allowed the detection of *Salicaceae* bud compounds with potential pharmacological activities, *e.g.* antibacterial and phytoestrogenic potential. Selective acetylcholinesterase inhibitors are interesting for the symptomatic treatment of Alzheimer's disease and some other dementias. Chemistry and bioactivity discrepancies were found between samples of *Populus nigra* (not between these and *P. alba*), making our method useful for the fast chemotypal determination of extracts. In the future, this hyphenated HPTLC method would also be easily adapted for quantitative control analysis, and for structural identification of bioactive molecules, by hyphenation to targeted high-resolution mass spectrometry. It is evident that progress in structural elucidation techniques at the analytical scale will be the next important advancement in HPTLC-EDA.

References [1] J.E. Eckenwalder, *Systematics and evolution of* Populus, in: R.F. Stettler, H.D. Bradshaw Jr., P.E. Heilman, T.M. Hinckley (Eds.), *Biology of* Populus *and its implications for management and conservation*, NRC Research Press (1996), pp. 7–32. [2] I. Jerković, J. Mastelić, Phytochem 63 (2003) 109–113. [3] G.E. Morlock *et al.*, CAMAG Bibliogr Serv 111 (2013) 13-15. [4] R. Akkad, W. Schwack, J Chromatogr B 878 (2010) 1337–1345. [5] A. Marston *et al.*, Phytochem Anal 13 (2002) 51–54.

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