# Effect-directed analysis of bioactive constituents of German propolis samples

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## Introduction

The propolis produced by European bee *Apis mellifera* L. is increasingly used in natural medicine or as health food, for various purposes, due to its numerous properties [1], *e.g.* anti-infectious, neuroprotective, antitumoral, anti-angiogenic, anticaries, *etc*. For effect-directed analysis (EDA), an HPTLC method hyphenated to several bioassays was developed to detect chemical constituents with pharmacological activity such as estrogenic and antibacterial chemical constituents, as well as inhibitors of acetylcholinesterase (AChE).

## Results and discussion

### *1. Extract preparation, separation and derivatization*

Analysis of 28 propolis sample extracts, obtained from various regions of Germany, was performed on HPTLC plates silica gel 60 (Fig. 1 A) with a mixture of hexane, ethyl acetate and acetic acid, under fuming HCl atmosphere [2]. For microchemical derivatization of phenolic compounds, Neu's reagent with polyethylene glycol 400 was used, showing five different general patterns at UV 254 nm and UV 366 nm (Fig. 1, B and C). For comprehensive information on the bioactivity profile of these samples, various bioassays were employed.

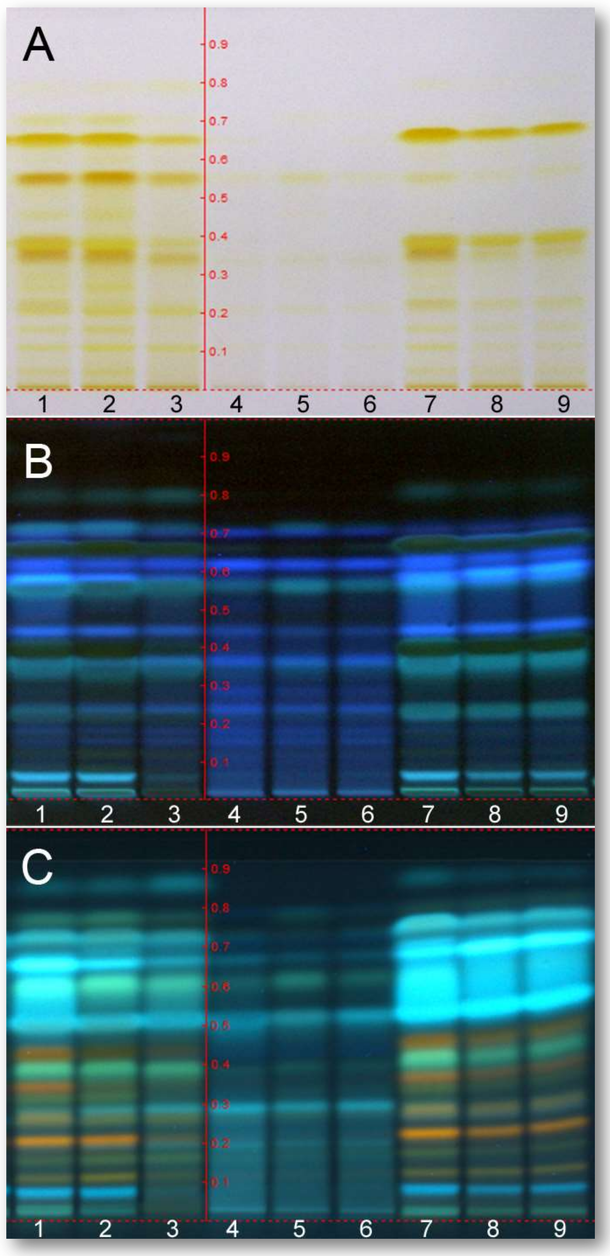


**Fig. 1** Chromatograms of propolis extracts (A) under white light and (B) at UV 366 nm before derivatization and (C) after derivatization with natural product (Neu's) reagent

### *2. Estrogenic activity*

The samples were submitted to the recently modified planar yeast estrogen screen (pYES), with which sharply-bounded zones were obtained [3, 4], for detection of components with (phyto)estrogenic activity. In the more apolar $hR_F$ range, (phyto)estrogenic compounds were discovered in each propolis sample (Fig. 2, D and E).

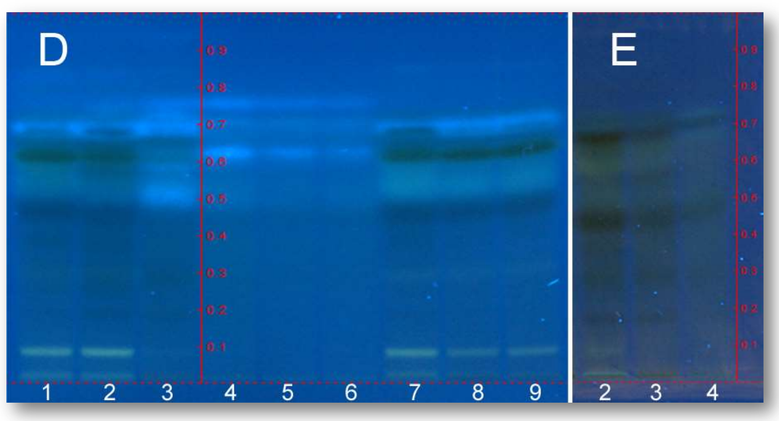


**Fig. 2** Chromatograms of propolis extracts at UV 366 nm after (D) pYES bioassay and (E) pYES bioassay without yeast (blank)

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### *3. Antimicrobial, Af-bioactive and AChE inhibiting activities*

Antimicrobial and bioactive components were detected using the *Bacillus subtilis* [5] and *Aliivibrio fischeri bioassays* (according to *DIN EN ISO 11348-1*), respectively. All samples contained at least 2 compounds with antimicrobial activity against *B. subtilis* (Fig. 3 F) and 5 *A. fischeri*-bioactive compounds (Fig. 3 G). The optimized [6] Marston's colorimetric method [7] was adapted and applied for the identification of AChE inhibitors. At least one inhibitor was found in each sample, some of them containing more (Fig. 3 H).

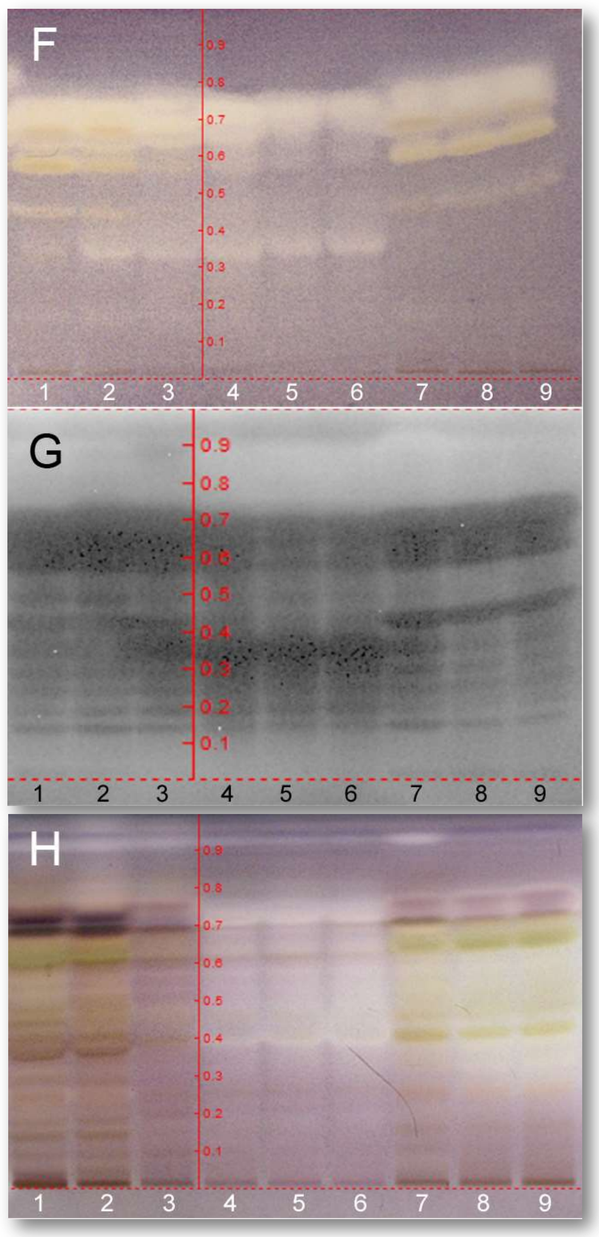


**Fig. 3** Chromatograms of propolis extracts under white light after (F) *Bacillus subtilis* and (G) *Aliivibrio fischeri* bioassays, and (H) the AChE enzyme inhibition assay

## Conclusion

Hyphenated HPTLC (with a parallel analysis of up to 20 samples on one plate) allowed the detection of propolis compounds with potential pharmacological activities, *e.g.* antibacterial and phytoestrogenic. Selective AChE inhibitors, interesting for the symptomatic treatment of Alzheimer's disease and some other dementias, were also found in the majority of the propolis samples. However, strong bioactivity discrepancies and chemical pattern variations were observed, too, reflecting the various origins of the samples and the importance of chemotypical determination; for this very purpose, our method will be useful, as it allows a fast qualitative assessment of the propolis accessions, which could be necessary to prevent unwanted side effects [8]. In the future, this hyphenated HPTLC method would also be easily adapted for quantitative control analysis, and for structural elucidation of bioactive molecules, by hyphenation to targeted high-resolution mass spectrometry and other advanced techniques at the analytical scale.

**References** [1] N. Cardinault *et al*. Phytothérapie 10 (2012) 298–304. [2] G.E. Morlock *et al.* CBS 111 (2013) 13-15. [3] I. Klingelhöfer, G.E. Morlock, J Chromatogr A 1360 (2014) 288-295. [4] G.E. Morlock, I. Klingelhöfer, Anal Chem 86 (2014) 8289–8295. [5] M. Jamshidi-Aidji, G.E. Morlock, International Symposium for HPTLC, Lyon, 2014, poster 55. [6] R. Akkad, W. Schwack, J Chromatogr B 878 (2010) 1337–1345. [7] A. Marston *et al.* Phytochem Anal 13 (2002) 51–54. [8] M.G. Miguel, M.D. Antunes, JPBS 3 (2011) 479–495.

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