Bioactivity profiling of fresh and dried elderberry (*Sambucus nigra* L.) fruit extracts

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Introduction

In recent years an increasing number of consumers ask for products with health promoting properties to mitigate so-called civilization diseases which are often related to oxidative stress. In addition to obvious products like fruits and vegetables, functional food attracts increasingly more attention. A source with potentially helpful natural compounds are the fruits of elder (*Sambucus nigra* L.). The purple-black berries are rich in anthocyanins, a subgroup of flavonoids, which are highly bioactive. Additionally, many health-promoting qualities are attributed, like being *e. g.* anti-inflammatory, antimicrobial, antioxidative and cancer preventive [1]. In many countries *S. nigra* is traditionally used due to their diaphoretic and diuretic effects, as well as for treating of various viral infections including the common cold and flu. In this study, fresh and dried samples were separated by HPTLC, followed by bioactivity profiling using the subsequent (bio)assays: DPPH*, *A. fischeri* (*Af),* planar yeast estrogen screening (pYES), acetylcholinesterase (AChE) und tyrosinase (Tyr). Further investigations for characterization of the anthocyanins and other bioactive zones were carried out by HPTLC-ESI-MS after zone-elution via the TLC-MS Interface.

Results and discussion

The fruits of six of the ten samples originated from wild growing populations from central part of Poland (Fig. 1, tracks 1-4, 9 and 10) and the other four from cultivated plants (Fig. 1, tracks 5-8). Extracts were prepared from berries prepared freshly (Fig. 1, F) as well as dried directly after harvesting (Fig. 1, D). For the quantitation and most of the (bio)assays (DPPH*, *Af*, AChE, Tyr), the separation was performed on HPTLC plates silica gel 60 with ethyl acetate – 2-butanone – formic acid – water (5:3:2:1). For pYES, the separation was done on HPTLC plates RP18 W with water – *n*-propanol – formic acid (18:8:0.1). The absorbance was measured at 540 and 560 nm via multi-wavelength scan.

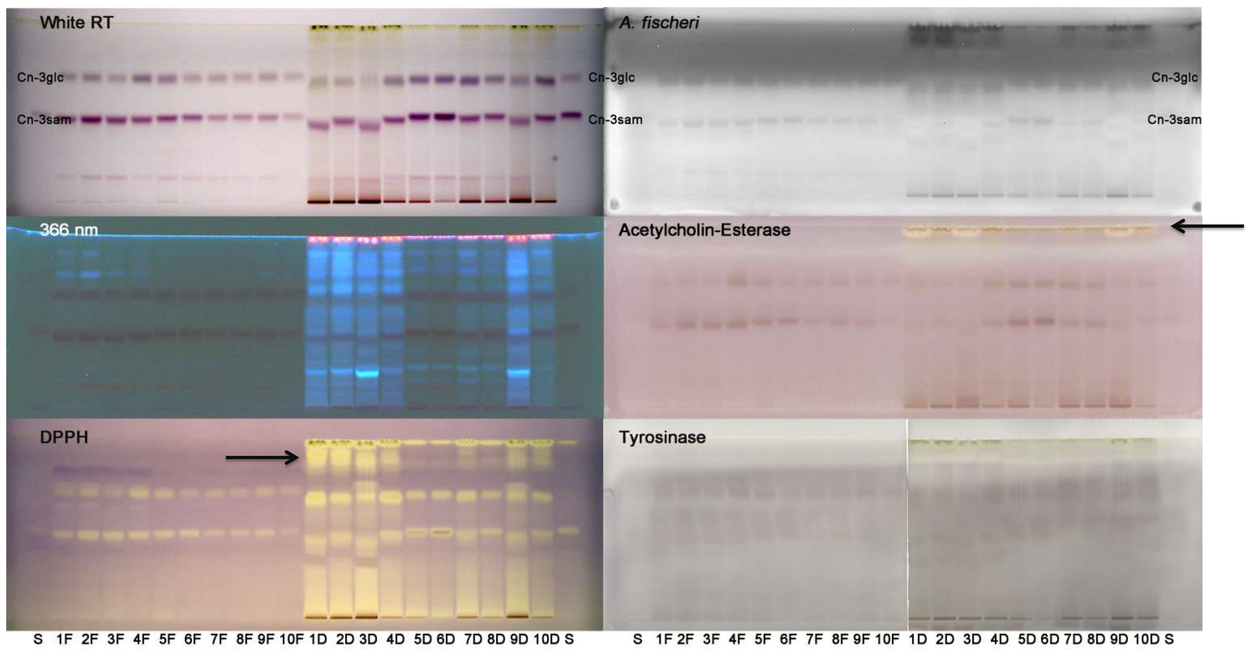

Fig. 1 HPTLC chromatograms of cyanidin-3-glucoside (cn-3-glc) and cyanidin-3-sambubioside(cn-3-sam) in fresh (1F-10F) and dried *S. nigra* samples (1D-10D) documented at white light illumination, UV 366 nm and after various (bio)assays

The coefficient of correlation for two different calibrations was 0.9939 and 0.9999. For the analysis of cn-3-glc (hR_F 68) and cn-3-sam (hR_F 48), the mean repeatabilities (*%RSD*, n=2) of fresh and dried samples were 1.1 % and 1.0 %, respectively. The intermediate (interday) precision was < 6.5 %. The anthocyanin content in the berries of the cultivated plants was up to three times higher compared to the content of the wild grown berries. Interestingly, from most of the dried samples (Fig. 1, tracks 4-10) a higher anthocyanin content was extracted than from the fresh berries (1.5 to 4 times higher), while the other dried samples (Fig. 1, tracks 1-3) showed a comparable or lower content.

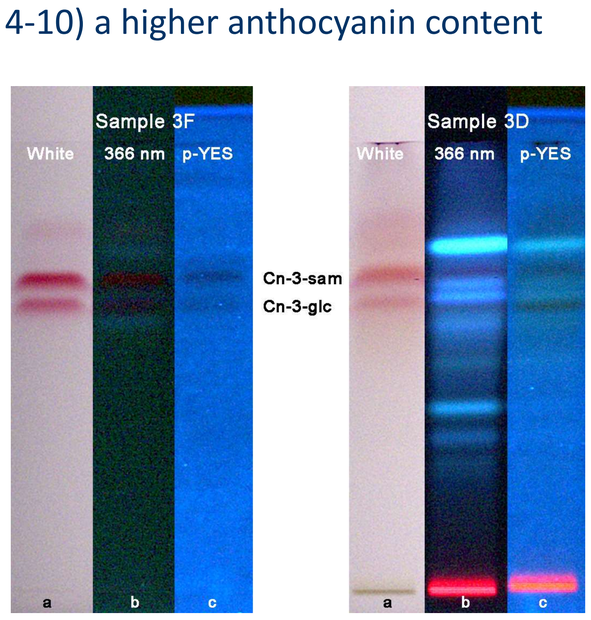

Fig. 2 HPTLC-pYES bioassay, exemplarily shown for the fresh and dried extracts of sample 3 (3F and 3D). Documentation at white light illumination (a) and at UV 366 nm, before (b) and after (c) the applied pYES bioassay. Negative results for both samples, also for the dried extract, were obtained due to the native fluorescence.

The separated anthocyanins showed a clear radical-scavenging activity (DPPH*) und a negative influence on *Af* (dark zones). Further substances in the dried samples showed a radical-scavenging activity and some were also indicated in the AChE assay (hR_F 100; black arrows). Neither with the Tyr inhibition assay (Fig. 1) nor pYES bioassay (Fig. 2) any bio-active compound linked to the corresponding effect could be identified.

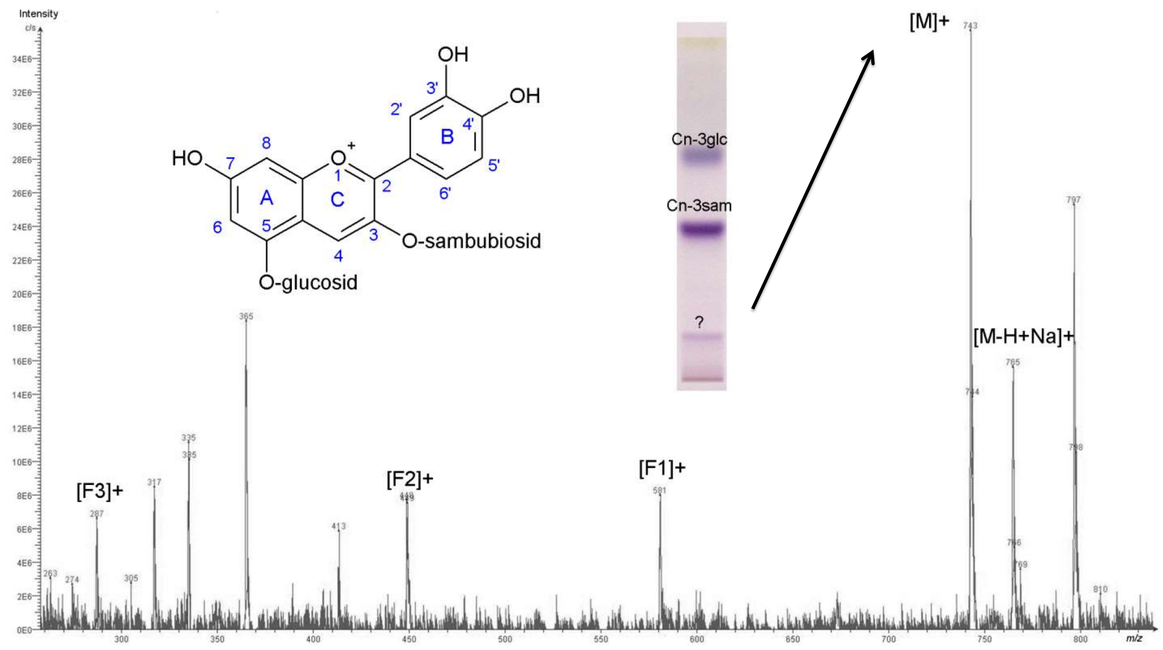

Fig. 3 HPTLC-ESI$^+$-MS spectrum of the unknown anthocyanin zone (hR_F 13) assigned as cn-3-sam-5 glc

In all extracts, cn-3-glc and cn-3-sam were the main anthocyanins found. Additionally, a faint lilac colored zone at hR_F 13 was visible in all samples. Due to the hR_F value, it was assumed that the unknown compound zone consisted of a multiply glycosylated anthocyanin. An anthocyanin that is commonly found in elderberry and multiply glycosylated is cn-3-sam-5-glc [2]. For the identification of the anthocyanin, the zone was investigated via HPTLC-ESI-MS. The spectra showed mass signals at *m/z* 278, 449 and 581, which could be assigned to the aglycon $[F3]^+$, the monosaccharide $[F2]^+$ and the disaccharide of cyanidin and cn-3-sam $[F1]^+$. The mass ions at *m/z* 743 and 765 corresponded with cn-3-sam-5-glc $[M]^+$ and its sodium adduct. The bioactive zones discovered in the solvent front (positive response with DPPH*-, AChE- and *Af*-assays) were investigated, too. The bioactive compound could be assigned to either oleanolic or ursolic acid.

Conclusion

The method was well suited for the quantification of anthocyanins from elderberry extracts. Moreover, the method allowed an easy and parallel investigation of all samples, not only concerning their anthocyanins but also other bioactive compounds discovered by various bioassays. Additionally, unknown bioactive zones were further characterized via HPTLC-ESI$^+$-MS, exemplarily shown for an anthocyanin zone.

Literature [1] J. Shipp, E.-S. Abdel-Aal, The Open Food Science Journal 4 (2010) 7-22. [2] J. Lee, C.E. Finn, J Sci Food Agric 87 (2007).

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