ABSTRACT

Analytical methods for the determination of phenolic compounds in propolis and plant materials were developed. Most of our research was focused on rhizomes of invasive alien plant species Japanese knotweed (*Fallopia japonica* Houtt.) to exploit their abundant biomass and explore other positive aspects.

We developed the HPTLC method for the analysis of phenolic acids, optimized the HPTLC method for the analysis of flavonoid aglycones and optimized conditions for densitometric and HPTLC–MS^n analyses of phenolic acids and flavonoids. Using these new methods we analysed propolis, roasted coffee, rose hip, hibiscus, rosemary and sage extracts. A multi-step TLC fractionation protocol on PLC silica gel and HPTLC silica gel or cellulose plates in combination with various developing solvents was developed to isolate phenolic compounds from 70% $acetone_{(aq)}$ extract of Japanese knotweed rhizome bark. We detected procyanidins B1 and B2, resveratrol-malonyl-hexoside, resveratrol-acetyl-hexoside, methyl derivatives of emodin bianthrone and emodin bianthrone-hexose, and taxifoline derivatives and isolated procyanidins B1 and B2 and emodin-*O*-malonyl glucoside for the first time in Japanese knotweed rhizomes. (+)-Catechin, (–)-epicatechin, (–)-epicatechin gallate, dimeric proanthocyanidin type B gallate, procyanidin B3, emodin and emodin-8-*O*-glucoside were also isolated. Isolated compounds were identified by HPTLC, HPTLC–MS^n and ^{1}H NMR (most compounds). Aliphatic contaminants originating from the HPTLC stationary phase and from laboratory consumables (leaching of the lubricant oleamide), were collected in isolates and their origins were confirmed by ^{1}H NMR and the HPTLC method for the separation of classes of lipid compounds. The UHPLC–ESI-MS method was developed for analyses of leachates and extracts of laboratory consumables, which were found to interfere with analytical and bioassay results.

We tested the pro-inflammatory and anti-inflammatory activity of 70% $ethanol_{(aq)}$ and 70% $acetone_{(aq)}$ extracts from Japanese knotweed rhizomes and rhizome bark via the TLR4 signalling pathways and for all detected pro-inflammatory activity at 100 μg mL^{-1}. A DPPH test was used to determine the antioxidant activity of Japanese knotweed rhizome bark extracts prepared with eight different solvents. All extracts gave low IC_{50} values (2.6–3.5 μg mL^{-1}) comparable to the IC_{50} value of ascorbic acid immediately after preparation (3.1 μg mL^{-1}). We developed a SEC-HPLC–UV method and performed *on-line* DPPH guided fractionation of 70% $ethanol_{(aq)}$ extract using post-column derivatization with the DPPH reagent. We also developed a RP-HPLC–UV–MS^n method, analyzed SEC fractions and identified (–)-epicatechin in the fraction with the antioxidant activity. (–)-Epicatechin showed stronger antioxidant activity (IC_{50} = 1.8 μg mL^{-1}) than the extracts and ascorbic acid. The antioxidant activity of (–)-epicatechin and the 70% $ethanol_{(aq)}$ extract remained stable for at least 14 days, while the antioxidant activity of ascorbic acid decreased. The 70% $ethanol_{(aq)}$ extract was incorporated in the chitosan foils. The transition of antioxidants from the foil to the contact fluid (food simulant) was confirmed, thereby laying the foundation for development of biodegradable containers for food/drink and pharmaceutic forms, which would protect the contents against oxidation.