Application of Matrix Solid-Phase Dispersion for HPLC analysis of polyphenol profile in 50-years old herbarium specimens of _Polygonum aviculare_ L.

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Introduction

Plant samples stored in herbaria can be very valuable for comparative studies in chemotaxonomy and diversity of medicinal plants as well as aid in proper identification of unknown material and voucher specimens stored in various depositories. Also, little is known about the stability of polyphenol compounds in plant material stored for decades or even centuries. However, herbaria specimens are under special protection and may not provide sufficient amounts of material for comprehensive analysis (Foutami et al. 2018). In this study, we employed Matrix Solid-Phase Dispersion (MSPD) procedures (Dawidowicz and Wianowska, 2009) to prepare extracts for HPLC analysis using as little as 100 mg of plant material from up to 57-years old samples of _Polygonum aviculare_ L.

Materials and methods

Plant material

The herbaria specimens were made available for sampling by two certified herbaria in Polish universities curating the series – _Flora Polonae Exsiccata_:

Adam Mickiewicz University – Faculty of Biology – index herbariorum code POZG (P);
Gdansk University – Department of Plant Taxonomy – code UGDA (G).

The specimens were collected mainly in Northern Poland between 1962-1977 from various habitats. We also used a commercial herb purchased from HerbaPol (Poland) as the pharmacopeial herb (PhEur) – dried _Polygoni avicularis herba_.

Extraction

The possibly representative samples were separated (using forceps) from intact parts of a specimen (leaves attached to tiny stems) and weighed accurately. Most of samples removed from a single specimen weighed about 300 mg. Then, they were ground in a mortar and pestle, weighed accurately to obtain portions of 100 mg (± 0.1) and extracted using the published Matrix Solid-Phase Dispersion Extraction (MSPDE) method (Dawidowicz & Wianowska, 2009). The parameters (were optimized for the commercial _Polygoni avicularis herba_ sample using surface-response methodology with maximized total polyphenol content as target. A routine solvent (aq.MeOH 80%) extraction of the commercial sample was used for profile comparison.

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HPLC-DAD

HPLC system: Agilent 1260 series with diode array detector (DAD);
Elution conditions: Phenomenex Kinetex C18 analytical column (150 mm x 3.0 mm, particle size: 2.6 µm). Mobile phase A [water:formic acid (100:0.1, v/v)] and mobile phase B [acetonitrile:formic acid (100:0.1, v/v)] with a multistep gradient: 0–2 min 10% B, 2–6 min 10–20% B, 6–11 min 20–50% B, 11–13 min 50–90% B, 13–14 min 90% B; flow rate: 0.4 ml/min. Injection volume 10.0 µl.

UV–Vis spectra were recorded in the range between 200 and 500 nm. The compounds were identified based on their tR and UV spectra.

Results and discussion

The one step extraction with C18 silica gel as dispersing phase in proportion of 1:4 of herbal material eluted with methanol was sufficient to obtain comparable and repeatable profiles not different from the classical solvent extraction. The major flavonoids such as several quercetin, myricetin, and kaempferol glycosides were well preserved in most of the 18 analyzed herbarium samples as compared to the freshly prepared material from validated Polygoni avicularis herba. 100 mg of plant material was enough for extraction and did not require significant damage to the specimens. The samples despite differences in collection years and classification to various subspecific taxa were all very similar in terms of the TLC and HPLC profiles and content of major flavonoids. The reference material from vast collections of herbaria has been insufficiently utilized as a source of phytochemical studies and elaboration of sparing and reliable extraction method is of crucial importance to comparative studies. It may be extremely helpful in solving various taxonomic, ecological and evolutionary problems in phytochemistry of medicinal plants (Foutami et al., 2018).

Conclusion

The tested method proves the concept of using MSPD as a convenient approach for extracting of unique historical samples or when only small amounts are available.

References
