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Extraction methods for the determination of phenolic compounds from *Equisetum arvense* L. herb

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ABSTRACT

Polyphenols are a very important group of pharmacologically active compounds. The extraction yield of selected phenolic acids and flavonoids from *Equisetum arvense* L. herb has been determined by use different methods of extraction – Soxhlet extraction, ultrasound assisted extraction (USAE), and accelerated solvent extraction (ASE). Methanol and 80% aqueous methanol were used as extractants. The crude extracts were evaporated to dryness and prepared for fractionation of the analyzed compounds by solid-phase extraction (SPE). An alternative method used for the isolation and purification of analyzed polyphenols was matrix solid-phase dispersion (MSPD). Samples containing the phenolic fraction were analyzed by RP-HPLC. Quantitative analysis was performed by the external standard method and use of a calibration plot for each standard. The most effective technique was ultrasound assisted extraction. Only for caffeic acid the highest yield gave exhaustive extraction in a Soxhlet apparatus. The methods were validated in terms of accuracy, precision LOD and LOQ.

1. Introduction

Equisetum arvense L. (horsetail), belonging to Equisetaceae family, is a plant showing aerial stems, branched with regular verticilies 2–23 mm in diameter, terminal strobile in the branches and in the main stem (10 mm long and 4 mm in diameter). This plant grows in several regions of Europe and North, Central, and South America (Dos Santos et al., 2005; Garcia et al., 2013).

Studies of *E. arvense* have reported on its antioxidant constituents (i.e. caffeic acid, chlorogenic acid, ferulic acid, kaempferol, quercetin, isoquercetin, apigenin, and luteolin) (Garcia et al., 2012). Moreover horsetail is rich in many kinds of vitamins, such as B1, B2, B6, nicotinic acid, folic acid, pantothenic acid, and vitamins C, E, K, silicic acid, saponins, and trace elements such as Na, K, Ca, Mg, P, Fe, Zn, Cu, Mn, Si, Sr, and Ti. In conclusion this medical plant may be useful to protect against the various diseases (Nagai et al., 2005). Horsetail has traditionally been used as a diuretic agent, and sometimes is suggested for the following diseases: kidney stones, urinary tract infections, brittle nails, minor wounds, and burns (applied topically) (Nagai et al., 2005).

The antioxidant activities of the plant extracts largely depend on the extraction efficiency of bioactive components, and composition of the extracts. The extraction yield of active compounds from plant materials is affected by different factors, such as the extraction techniques, solvents, time, temperature, solvent-to-plant material ratio, and many others. However, a suitable extracting method, and solvent are crucial to ensuring an efficient extraction of the targeted nutraceuticals from plant material (Karabegović et al., 2014).

Various solvents, such as methanol, ethanol, acetone, ethyl acetate, and their combinations have been used in previous studies for the extraction of phenolic compounds from plant materials, often with different proportions of water, in order to establish their extractive efficiency (Nour et al., 2014; Dai and Mumper, 2010; Fernandez-Agullo et al., 2013; Vongsak et al., 2013). The extraction of these compounds may be performed by traditional or by modern methods (such as: accelerated solvent extraction (ASE), ultrasound assisted extraction (USAE), microwave assisted solvent extraction (MASE) etc. The novel methods require shorter extraction time, use of low amount of solvents, allow for simultaneous parallel processing of several samples, and are automatic, but are more expensive. The important step in sample preparation is

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clean-up of crude extract. The most used purification technique is the solid-phase extraction (SPE) (Chen et al., 2008).

An alternative, introduced for sample preparation is matrix solid-phase dispersion (MSPD) (Capriotti et al., 2010; Visnevschi-Necrasov et al., 2009; Barker, 2000; Oniszczuk et al., 2013). It is a simple, and cheap preparation technique that combines sample homogenization, extraction, and purification of the analyzed compounds in one step (Barker, 2007; Kristenson et al., 2006; Dopico-Garcia et al., 2007). MSPD may selectively elute a single compound, several classes of compounds or fractions (Capriotti et al., 2010; Garcia-Lopez et al., 2008). This method can eliminate many complicated steps in classical liquid–solid extraction (LSE) and/or solid phase extraction (SPE), allows for the reduction of organic solvent consumption, exclusion of sample component degradation, improvement of extraction efficiency, and selectivity (Abhilash et al., 2007; Dawidowicz and Rado, 2010; Dawidowicz et al., 2011).

Theoretically, the optimal extraction method should be simple, safe, reproducible, inexpensive, and suitable for industrial application (Vongsak et al., 2013).

The aim of this paper was comparison of ultrasound assisted extraction, accelerated solvent extraction, Soxhlet extraction, and matrix solid-phase dispersion in the determination of selected phenolic compounds from *E. arvense* by HPLC-DAD.

In case of all liquid–solid extraction methods solid-phase extraction for the purification of crude extracts was used.

2. Materials and methods

2.1. Materials and chemicals

Field horsetail (*E. arvense* L.) herb was purchased from "*Flos*" herbal industrial (Mokrsko, Poland). Before the extraction dry plant material was milled, and sieved.

All standards were purchased from Sigma Aldrich (Steinheim, Germany). HPLC-grade methanol, acetonitrile, and formic acid were purchased from J.T. Baker (Netherlands), water was purified using a Millipore laboratory ultra pure water system (Simplicity™ system, Millipore, Molsheim, France). Methanol used for preparation, and purification of the extracts was of analytical grade, and obtained from the Polish Reagents (POCH, Gliwice, Poland). The solid phase used for MSPD was Alltech bulk high capacity C 18 sorbent, 50 μM (Alltech, Deerfield, IL, USA), end-capped, 17% C. The columns used for SPE were Bakerbond C 18, 3 mL columns, packed with 500 mg reversed phase, 40 μM (J.T. Baker, Deventer, Netherlands), end-capped, 17.5% C.

2.2. Sample preparation

2.2.1. Soxhlet extraction

2 g of dried *E. arvense* herb powder was placed to the filter paper, and extracted with applicable solvent (methanol or 80% aqueous solution of methanol) for 48 h in Soxhlet apparatus. Extracts were evaporated to dryness. The residues were dissolved in 25 mL of 80% aqueous solution of methanol. The whole procedure was repeated three times for each solvent.

2.2.2. Ultrasound assisted extraction (USAE)

2 g of dried *E. arvense* herb powder was extracted with 50 mL of applicable solvent (methanol or 80% aqueous solution of methanol) in ultrasonic bath with temperature regulation (Bandelin Electronic, Sonorex RK 100H, Germany) at 60 °C for 30 min. Extract was filtered, and plant material was extracted afterwards with two portions of the solvent by the same way. Extracts were filtered, combined, and evaporated to dryness. The residues were dissolved in 25 mL of 80% aqueous solution of methanol. The whole procedure was repeated three times for each solvent.

2.2.3. Accelerated solvent extraction (ASE)

ASE was performed with a Dionex (Sunnyvale, CA, USA) ASE 200 instrument with solvent controller. Plant material (2 g) was placed in a stainless-steel extraction cell, and extracted with applicable solvent (methanol, and 80% aqueous solution of methanol). The extractions were performed at three temperature ranges (80, 100, and 120 °C) for 30 min (three cycles for 10 min at the same temperature, for every sample) at a pressure of 60 bar. Extracts were combined, and evaporated to dryness. The residues were dissolved in 25 mL of 80% aqueous solution of methanol. The whole procedure was repeated three times for each solvent.

2.2.4. Solid phase extraction (SPE)

All crude extracts (obtained by Soxhlet, USAE, and ASE) were purified by SPE. 10 mL of every sample was passed through a Bakerbond C 18 SPE column (previously conditioned). The retained polyphenols were eluted with 15 mL of 80% aqueous solution of methanol using a SPE vacuum. Fractions for analysis were transferred into volumetric flask, filled up to their volume with 80% aqueous solution of methanol, and injected into the HPLC system. The whole procedure was repeated three times for each solvent.

2.2.5. Matrix solid-phase dispersion (MSPD)

0.25 g of dried *Equisetum* herb powder was placed in a glass mortar, and mixed with 0.5 g of sorbent (previously conditioned), and 1 mL of 80% aqueous solution of methanol. The mixture was then homogenized in the glass mortar using a pestle to obtain a homogenous mixture. The blend was then transferred into a 3 mL syringe with a paper frit on the bottom. The sample was covered with another paper frit, and compressed using the syringe plunger. Polyphenols were eluted with 5 mL of 80% aqueous solution of methanol. Fractions for analysis were transferred into volumetric flask, filled up to their volume with 80% aqueous solution of methanol, and injected into the HPLC system. The whole procedure was repeated three times for each solvent.

2.3. HPLC conditions

Analysis was performed with a liquid chromatograph equipped Elite LaChrom VWR Hitachi DAD L-2455 (column oven Elite LaChrom L-2300, Autosampler Elite LaChrom L-2200, Pump Elite LaChrom L-2130), on the Merck LiChrocart 250-4 HPLC column (250 mM × 4.0 mM) packed with 5 μm (LiChrosper 100 RP-18 end-capped). The sample injection volume was 10 μL. The mobile phase was acetonitrile (A), and water containing 0.5% formic acid (B). The A% was changed as follow: 0 min (10%); 25 min (20%); 45 min (30%); 47 min (100%). The flow rate was 1 mL/min, the column temperature was 25 °C. The identification was performed by comparing retention times, and UV-DAD spectra with those analyzed under the same conditions for appropriate standards. The qualitative, and quantitative determination was performed in following wavelengths: λ = 255, and 323 nM.

The flavonoids, and phenolic acids content in the extracts were determined by use of calibration plots constructed for every standard. Standard solutions were prepared by dissolving the compounds in methanol.

2.4. Validation of the methods

The methods were validated in terms of accuracy, precision LOD, and LOQ. Moreover, the linear ranges of calibration curves were determined.