HPLC-ESI-TOF-MS CHEMICAL CHARACTERIZATION OF COMFREY ROOT EXTRACT OBTAINED BY SUBCRITICAL WATER EXTRACTION

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Abstract

In this work, a study on phytochemical profiles of comfrey (*Symphytum officinale* L.) root extract obtained by subcritical water extraction (SWE) has been carried out. Chemical composition was assessed by high-performance liquid chromatography coupled with electrospray time-of-flight mass spectrometry (HPLC-ESI-TOF-MS) identifying 22 compounds including organic acids, phenolic acids, flavonoids and fatty acids. Great number of phenolic acids and flavonoids were found in the extract obtained by SWE, with citric acid, caffeic acid and derivative, salvianolic acid B, hydroxybenzoic acid, syringetin-3-*O*-glucoside and quercetin 3-*O*-malonylglucoside as the most abundant compounds. Moreover, quercitin-3-malonylglucoside isomers, hydroxybenzoic acid glucoside, cirismaritin isomers, *p*-coumaric acid, hydroxycoumarin and methylcoumarin, among others, were identified for the first time in *S. officinale* root. Overall, the results indicate the potential of SWE for the production of high-quality plant extracts from *S. officinale* root.

Introduction

The global herbal medicine market grows continuously due to lifestyle change and scientific evidence of the beneficial health effects of herbs. Although traditional medicine represents the primary health care for around 80% of the total world population, mainly in the developing countries, the high potential of biologically active plant species remains largely unexplored [1]. In traditional medicine comfrey (*Symphytum officinale* L.) roots are used for the external treatment of joint disorders and musculoskeletal injuries of all kinds [2,3], while its internal use as infusions is very important in the treatment of gastro-intestinal and respiratory tract diseases [4]. The therapeutic properties of *S. officinale* are based on its anti-inflammatory and analgesic effects as well as its activity in stimulating granulation and tissue regeneration. *S. officinale* leaves and roots as the most used parts of this plant, contain allantoin, carotene, hydroxycinnamic acid derivatives, essential oils, vitamin B12 and zinc, which may be responsible for its healing properties.

For numerous plants, there is firm scientific evidence supporting their bioactivities, however traditional methods of preparation often do not use their full potential. In last decades, subcritical water extraction (SWE) attracts lots of attention due to its environmentally friendly character, low price, competitive solvating properties and exceptional selectivity. This technique relying on heated and pressurized water improve extraction efficiency, among others, due to lower viscosity of the solvent and consequently better penetration into the pores of solid particles. SWE is advanced extraction technique that reduces or eliminates the use of organic solvents, being suitable to produce safe pharmacologically active plant extracts and formulations. Therefore, the objective of this study was to carry out SWE for recovery of bioactive compounds from *S. officinale* root and to perform their characterization by high-performance liquid chromatography coupled with electrospray time-of-flight mass spectrometry (HPLC-ESI-TOF-MS).

Experimental

The commercial samples of dry *S. officinale* roots were purchased from local healthy food retail store in Novi Sad, Serbia. The roots were finely grounded and kept at room temperature and darkness until use.

SWE was performed in a house-made subcritical water extractor. Extraction procedure and apparatus were described previously [5]. Total capacity of high-pressure stainless-steel vessel was 1.7l. Pres- surization of the vessel was performed with nitrogen to prevent possible oxidation. Sample to distilled water ratio was 1:10. Extraction temperature (130°C) and extraction pressure (60 bar) were investigated as independent variables, while all other parameters were held constant (agitation rate of 2 Hz and extraction time of 20 min). After extraction, the vessel was cooled and depressurized. Obtained extract was filtrated and stored in a dark place at 4 °C until analysis.

Chemical profile of bioactive compounds from *S. officinale* root extract was defined using an Agilent 1200-HPLC system (Agilent Technologies, Palo Alto, CA, USA) of the Series Rapid Resolution coupled to an electro-spray time-of-flight mass spectrometer (HPLC-ESI-TOF-MS), previously described by García-Salas et al. [6] with some modifications.

Results and discussion

S. officinale root extract obtained SWE was analyzed by HPLC-ESI-TOF-MS (Figure 1). The identification of the compounds was based on MS spectra interpretation and considering data previously reported in the literature.

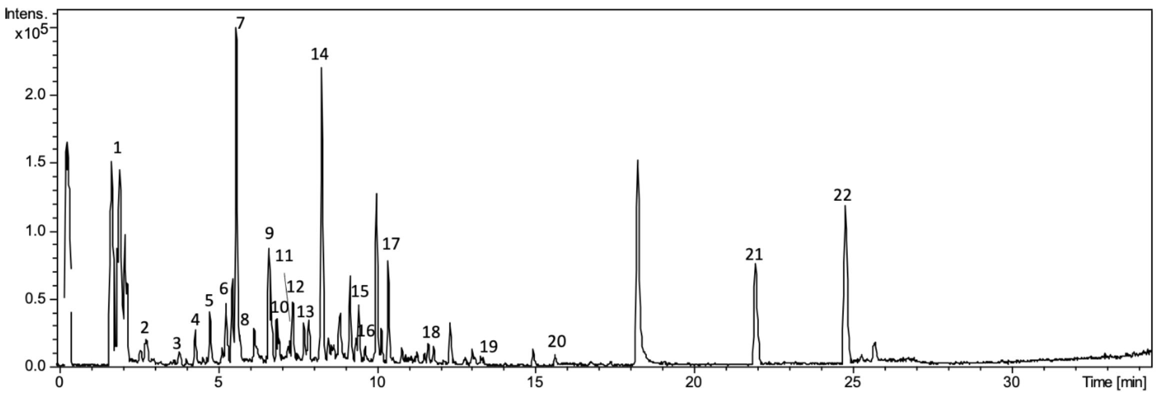

Figure 1. Base peak chromatogram of SWE *S. officinale* root extract obtained by HPLC-ESI-TOF-MS.

Table 1 lists the main peaks detected according to increasing retention time together with experimental and calculated *m/z*, and molecular formula. A total of 22 compounds were tentatively identified in *S. officinale* root belonging to various metabolite families that included anthraquinones, organic, phenolic and fatty acids, and their derivatives. Some of these compounds have been previously reported in *S. officinale* root, however, the TOF-MS enabled identification of 13 phytochemicals that have never been reported in this sample matrix.

Table 1. Proposed compounds tentatively identified in *S. officinale* root extract obtained by SWE using HPLC-ESI-TOF-MS. Numbers designing compounds correspond to peaks as depicted in Figure 1.

Peak	Retention time (min)	*m/z* experimental	*m/z* calculated	$(M-H)^-$	Proposed compound
1	1.98	377.0876	377.0878	$C_{18}H_{17}O_9$	caffeic acid derivative
2	2.33	191.0195	191.0197	$C_6H_7O_7$	citric acid
3	3.85	549.0876	549.0866	$C_{24}H_{21}O_{15}$	quercitin-3-malonylglucoside isomer 1*
4	4.33	549.0875	549.0866	$C_{24}H_{21}O_{15}$	quercitin-3-malonylglucoside isomer 2*
5	4.79	137.0245	137.0244	$C_7H_5O_3$	hydroxybenzoic acid isomer 1
6	5.15	151.0406	151.0401	$C_8H_7O_3$	hydroxyphenylacetic acid*
7	5.6	179.0358	179.035	$C_9H_7O_4$	caffeic acid
8	5.72	313.9725	313.0718	$C_{17}H_{13}O_6$	cirsimaritin isomer 1*
9	6.64	163.0411	163.0401	$C_8H_7O_3$	*p*-coumaric acid*
10	6.88	313.0735	313.0718	$C_{17}H_{13}O_6$	cirsimaritin isomer 2*
11	7.24	271.0971	271.0976	$C_{16}H_{15}O_4$	dihydroxy-dimethoxy-dihydrophenanthrene*
12	7.38	137.0245	137.0244	$C_7H_5O_3$	hydroxybenzoic acid isomer 2
13	7.73	219.0665	219.0663	$C_{12}H_{11}O_4$	dimethoxy-methylcoumarin*
14	8.30	507.1144	507.1144	$C_{23}H_{23}O_{13}$	syringetin-3-*O*-glucoside*
15	9.47	717.1445	717.1461	$C_{36}H_{29}O_{16}$	salvianolic acid B
16	9.65	311.0561	311.0561	$C_{17}H_{11}O_6$	acetyl-monomethyl-trihydroxy anthraquinone
17	10.15	161.0247	161.0244	$C_9H_5O_3$	hydroxycoumarin*
18	11.64	159.046	149.0452	$C_{10}H_7O_2$	methylcoumarin*
19	13.33	137.0241	137.0244	$C_7H_5O_3$	hydroxybenzoic acid isomer 3
20	15.59	329.2344	329.2333	$C_{18}H_{33}O_5$	trihydroxy-octadecenoic acid
21	21.88	221.1542	221.1547	$C_{14}H_{21}O_2$	di-tert-butyl-o-hydroquinone*
22	24.67	205.1596	205.1598	$C_{14}H_{21}O$	di-tert-butyl-phenol*

* Compound identified in *S. officinale* root for the first time.

Conclusion

HPLC-ESI-TOF-MS analysis was used to analyze the chemical profile of *S. officinale* root extract obtained by SWE. In analyzed extract, 22 comfrey metabolites were separated and identified, including 13 newly discovered compounds. Moreover, the most abundant compounds detected by HPLC-ESI-TOF-MS were citric acid, caffeic acid and derivative, salvianolic acid B, hydroxybenzoic acid, syringetin-3-*O*-glucoside and quercetin 3-*O*-malonylglucoside. According to the results, subcritical water as a cost-effective and green solvent has a great potential in exploitation of natural sources of bioactive compounds and production of functional and pharmacologically-active fractions. The present study also highlights the potential application of *S. officinale* root extract as constituents of new added-value formulations.

Acknowledgements

This work was supported by the Serbian Ministry of Education, Science and Technological Development (451-03-68/2022-14/200134).

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