

IDENTIFICATION OF BIOACTIVE COMPOUNDS IN COMFREY ROOT EXTRACTS

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Abstract

In the present study bioactive compounds present in comfrey root extracts obtained by supercritical fluid (SFE) and pressurized liquid extraction (PLE) were identified. Chemical characterization of the extracts was carried out by high-performance liquid chromatography coupled to DAD and electrospray-ionization time-of-flight mass spectrometry (HPLC–ESI–TOF–MS) yielding in total of 23 identified compounds. PLE as a fast, green and innovative approach, seems to be the best choice for extracting wide variety of compounds with different polarities within the shortest extraction time being the fatty acids and their derivatives the most abundant. The present study also highlights the potential application of comfrey root extracts as constituents of new added-value formulations.

Introduction

Comfrey (*Symphytum officinale* L.) is a medicinal plant widely spread across Europe, but it can also be found in some parts of Asia and South America. Pyrrolizidine alkaloids in comfrey have been linked to cases of hepatotoxicity and carcinogenicity, and for this reason in traditional medicine comfrey roots are used topically, mostly for the treatment of wounds, joint disorders, and musculoskeletal injuries of all kinds [1,2]. The content of pyrrolizidine alkaloids is the highest in comfrey root [3,4]. Active compounds identified in comfrey root include allantoin, rosmarinic acid and other hydroxycinamon acid derivatives, mucopolysaccharides, A, B and C vitamins, triterpenoid saponins, tannins, calcium, potassium and selenium [5,6]. Allantoin, as a principal compound identified in comfrey root, activates metabolic processes in subcutaneous tissue and stimulates the cell growth resulting in epithelization. It also strongly promotes the cell growth in bone cells and connective tissue [7].

In the literature only few papers deal with the extraction of bioactive compounds from comfrey root relying mostly on conventional solid/liquid extraction [8-11]. Conventional extraction techniques, however, are quite laborious, time- and solvent-consuming. The issues encountered in conventional extraction approaches can be overcome by application of modern extraction techniques.

In view of the fact that there is limited information available on the detailed chemical composition of comfrey root, present research was focused on the recovery of bioactive compounds using supercritical fluid (SFE) and pressurized liquid extraction (PLE). SFE and PLE have been compared in terms of their selectivity and efficiency to recover bioactive compounds from comfrey root. Major bioactive compounds in comfrey root were identified by high-performance liquid chromatography coupled to electro-spray time-of-flight mass spectrometry.

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.

The PLE experiment was performed in a static mode (1500 psi and 20 min as the pressure and extraction time) with 85% ethanol at the temperature of 63°C. The dried comfrey root sample (3 g) was mixed with 6 g of sand and loaded into a stainless-steel extraction cell. After that, the extraction conditions described above were applied and the extract was collected in vials. The residual solvent was evaporated. Dried extract was stored at -20°C and protected from light until analysis.

SFE-CO₂ extraction was performed at 40°C in a dynamic mode with CO₂ plus ethanol (7%) and pressure of 150 bar. For extraction, 5 g of comfrey root powder were mixed with sea sand, placed in the extraction cell and pressurized with CO₂. The total extraction time was established at 120 min for each experiment. The collected extract was concentrated in a water-bath at 40°C using a rotary evaporator. Dried extract was stored at -20°C and protected from light until analysis.

Chemical profile of bioactive compounds from comfrey root extracts 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. [12] with some modifications.

Results and discussion

Comfrey root extracts obtained by SFE and PLE were characterized by means of HPLC-ESI-TOF-MS. The compounds were tentatively identified on the basis of their MS spectra and the molecular formula provided by the software together with data previously reported in literature. The resulting base peak chromatograms of a representative comfrey root extract are presented in Figure 1.

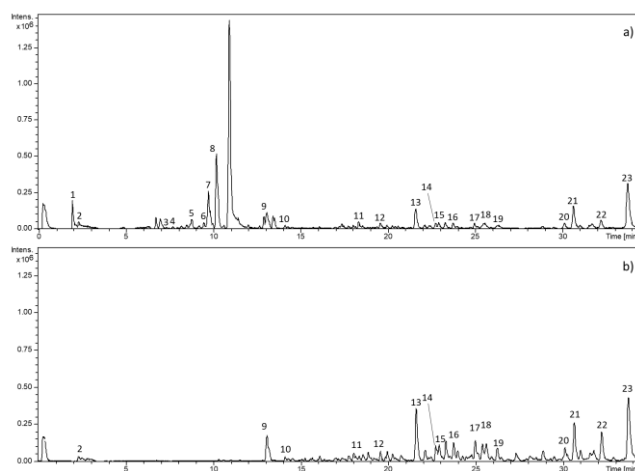


Figure 1. Base Peak chromatogram (BPC) of *S. officinale* root extracts obtained by: a) PLE and b) SFE. Peaks are numbered according to their elution order.

The chromatographic profile of *S. officinale* extract obtained by PLE (Figure 1a) showed the largest number of identified bioactive compounds, while the extract obtained by SFE (Figure 1b) showed the presence of more non-polar compounds as expected. A total of 23 compounds were identified in *S. officinale* root. Identified compounds belonged to different chemical

classes that included organic acids, phenolic compounds (simple phenols and antraquinones), and fatty acids and derivatives.

Table 1. Characterized compounds in comfrey root extracts obtained by SFE and PLE using HPLC-ESI-TOF-MS.

Peak	Retention time (min)	<i>m/z</i> experimental	<i>m/z</i> calculated	(M-H) ⁻	Proposed compound
1	2.01	377.0873	377.0878	C ₁₈ H ₁₇ O ₉	caffeic acid derivative
2	2.39	191.0195	191.0197	C ₆ H ₇ O ₇	citric acid
3	7.53	137.0234	137.0244	C ₇ H ₅ O ₃	hydroxybenzoic acid
4	7.92	179.0328	179.035	C ₉ H ₇ O ₄	caffeic acid
5	8.72	537.1033	537.1038	C ₂₇ H ₂₁ O ₁₂	salvianolic acid H/I
6	9.52	717.1475	717.1461	C ₃₆ H ₂₉ O ₁₆	salvianolic acid B
7	9.65	311.0562	311.0561	C ₁₇ H ₁₁ O ₆	acetyl-monomethyl-trihydroxy antraquinone
8	10.23	719.1624	719.1618	C ₃₆ H ₃₁ O ₁₆	sagerinic acid
9	13.09	329.2329	329.2333	C ₁₈ H ₃₃ O ₅	trihydroxy-octadecenoic acid isomer 1
10	13.99	329.2335	329.2333	C ₁₈ H ₃₃ O ₅	trihydroxy-octadecenoic acid isomer 2
11	18.55	311.2222	311.2228	C ₁₈ H ₃₁ O ₄	hydroperoxy-octadecadienoic acid
12	19.57	315.2551	315.2541	C ₁₈ H ₃₅ O ₄	dihydroxystearic acid
13	21.55	295.2283	295.2279	C ₁₈ H ₃₁ O ₃	hydroxy-octadecadienoic acid isomer 1
14	22.63	293.2125	293.2122	C ₁₈ H ₂₉ O ₃	oxo-octadecadienoic acid isomer 1
15	22.84	293.2126	293.2122	C ₁₈ H ₂₉ O ₃	oxo-octadecadienoic acid isomer 2
16	23.78	293.2125	293.2122	C ₁₈ H ₂₉ O ₃	oxo-octadecadienoic acid isomer 3
17	25.12	295.2275	295.2279	C ₁₈ H ₃₁ O ₃	hydroxy-octadecadienoic acid isomer 2
18	25.58	295.2289	295.2279	C ₁₈ H ₃₁ O ₃	hydroxy-octadecadienoic acid isomer 3
19	26.12	295.2271	295.2279	C ₁₈ H ₃₁ O ₃	hydroxy-octadecadienoic acid isomer 4
20	30.06	277.2176	277.2173	C ₁₈ H ₂₉ O ₂	linolenic acid isomer 1
21	30.59	277.2188	277.2173	C ₁₈ H ₂₉ O ₂	linolenic acid isomer 2
22	32.15	253.2177	253.2173	C ₁₆ H ₂₉ O ₂	palmitoleic acid
23	33.68	279.2339	279.233	C ₁₈ H ₃₁ O ₂	linoleic acid

Conclusion

The present study aimed to compare extraction of bioactive compounds from comfrey root using PLE and SFE for recovery of natural constituents with interest in food, pharmaceutical and cosmetic industries. A potent HPLC method coupled to DAD and TOF-MS has been used to characterize comfrey root extracts, allowing identification of 23 compounds. The main compounds detected in the extracts were identified as phenolic acids (mainly sagerinic acid), and fatty acids (especially linolenic and linoleic acid). The application of PLE proved to be more advantageous comparing to SFE allowing extraction of wide variety of compounds with different polarities within the shortest extraction time. The present study also highlights the potential application of *S. officinale* extracts as a source of diverse bioactive compounds to design new functional foods, nutraceuticals and cosmetic products.

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