

Medicinal Herb Extraction Strategy – A Solvent Selection and Extraction Method Study

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Abstract

Medicinal herbs have a long history of use in improving human health and curing diseases. Herbal materials are naturally an excellent medicine library because they provide unlimited components with complex chemical structures and a wide range of bioactivities. Herbal materials have thus attracted wide interest of researchers in the identification of the active components and verification of their efficacy. Herb preparation and extraction is the first step and in fact one of the most important steps in the chemical identification process. This step makes the down stream processes possible, including bioactive component identification, isolation and characterization.

In this research, using a model herb material, Echinacea, we studied chemical extractability of different solvents with various polarities. Several extraction methods were used and their extraction efficiencies were compared. The extraction methods used in this research were soxhlet extraction (SE), sonication assisted extraction (SAE), and accelerated solvent extraction (ASE). Chemical profile and yield of each extract and the throughput capability of different extraction methods were systematically compared and discussed. Based on the results, a recommendation was made for a consolidated herb extraction-fractionation process.

Introduction

Evidence based activity is the key for the development of modern herbal products. Herb preparation and extraction is the first step and in fact one of the most important steps in the bioactivity and chemical identification process. This step makes the down stream processes possible, including bioactive component identification, isolation and characterization. There are several extraction techniques that are commonly used, including decoction, steam distillation, solvent extraction (soaking and leaching), soxhlet percolation, ultrasound assisted extraction (UAE), microwave assisted extraction (MAE) pressurized solvent extraction (PSE), and the combinations of two or more techniques mentioned above.¹⁻⁴

Considering operational cost and scale-up possibilities, soxhlet extraction (SE), sonication assisted extraction (SAE), and accelerated solvent extraction (ASE, also known as PSE) were selected for comparison in this study. Both sequential and parallel extraction techniques were used in this study. Chemical profiles of each solvent extract, extraction yield and the throughput capability of the different methods were the focus of this study.

Solvents used in herb extraction need to be volatile and leave no residue when dried. This solvent property will make the downstream processing easier, including solvent exchange and bioassay. Hexane, chloroform, ethyl acetate, methanol or ethanol are thus proper candidates and are widely used in herb processing. In this study water and DMSO were also selected due to their popularity in animal studies and bioassay.

Materials and Methods

Materials

Echinacea angustifolia was obtained from Nutrilite, CA. Solvents used for extractions, including hexane, chloroform, ethyl acetate, methanol, reagent alcohol (a mixture of ethanol, methanol and isopropanol), DMSO and water are of HPLC grade. DMSO, reagent alcohol and water were also used as a mixture at 5/3/2 by volume, so called DEW.

ASE Extraction method and procedure

In a “sequential extraction” technique, the residue of the previous extraction is used as the feed for the next extraction. The extraction temperature was 70 °C for hexane, chloroform, and ethyl acetate, 85 °C for methanol, 100 °C for DMSO/methanol/water, and 120 °C for water. The operation pressure was also monitored.

For each extraction step (each solvent), there was a static extraction stage (soaking) and a dynamic extraction stage (flowing through). The static extraction time is about 20 minutes, and the dynamic extraction time is also 20 minutes at a flow rate of 6 Bed Volume/hour. There were two static/dynamic extraction cycles in each solvent extraction step. Before switching to the next solvent, the sample bed was purged with air to avoid carryovers and contaminations. The purged liquid was combined with the extracts.

Soxhlet extraction and sonication assisted extraction (SAE)

Powdered botanicals were extracted at a solvent to mass ratio of 10/1 (v/w). Both sequential and parallel techniques were used in the soxhlet extraction. In SAE process, an individual sample was directly extracted by first soaking it overnight while stirring and followed by sonication for 1 h at room temp. The extract was then filtered out.

Yield measurement

The extract solutions of hexane, chloroform, ethyl acetate, and methanol were vacuum dried with rotary evaporator. The final extract weight was measured in a pre-weighed flask.

HPLC analysis

Two different HPLC conditions were used to analyze the extract samples. The first condition used a Luna PFP column (3 μ m, 150 mm x 4.6 mm) with a 0.2% phosphoric acid/acetonitrile gradient starting at 5/1. The second condition used a Synergi MAX-RP column (4 μ m, 150 mm x 4.6 mm) with a water/acetonitrile gradient starting at 6/4.

Experimental Results

Four extraction methods were used in this study, including soxhlet, sonication assisted extraction, and ASE. The extracts were then dried by rotary evaporation. Extraction solvents were arranged in sequential or parallel scheme. The dry mass was measured and the extraction yield was calculated. The results were recorded in Table 1. In this study, yield is defined as ratio of extract dry mass to raw material mass.

Table 1. Extraction yield of different techniques using different solvents. (mg extract / g raw material)

| Solvents\Technologies | Soxhlet -sequential | Soxhlet -parallel | SAE -parallel | ASE -sequential |
|-----------------------|------------------------|----------------------|------------------|--------------------|
| Hexanes | 3.26 | 19.5 | 10.6 | 15.6 |
| Chloroform | 13.27 | 31.5 | 18.8 | 8.7 |
| Ethyl Acetate | 1.94 | 32.7 | 17.6 | 2.6 |
| Methonal | 84.8 | 197.5 | 115 | 124.9 |
| DEW(DMSO/ROH/water) | - | - | 190 | 125.4 |
| Water | - | - | - | 144.6 |

Chromatographic profiles

The dried extracts were re-suspended in methanol at a nominal concentration of 5mg/mL. The clear solution part was used for HPLC analysis. The HPLC chromatograms of extracts generated using different technologies were recorded in Figure 1, and 2. Only the chromatograms of chloroform and methanol extracts were shown. When the same solvent was used, the chromatograms were very similar, in spite of the different processes or extraction methods.

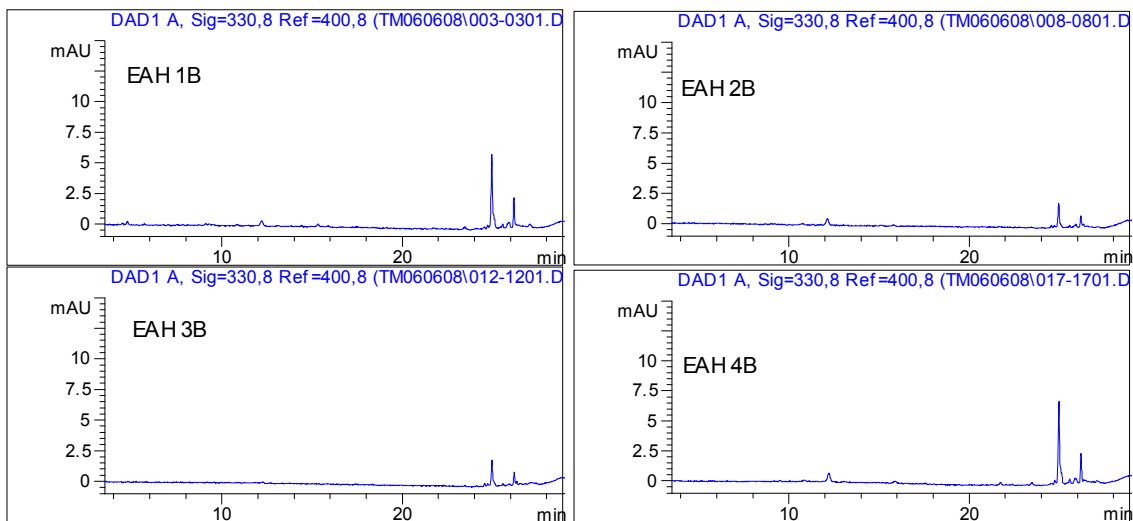


Figure 1. HPLC profiles of chloroform extract. The extraction methods used were, EAH1B, soxhlet-sequential; EAH2B, soxhlet-parallel; EAH3B, vortex-sonication; EAH4B, ASE.

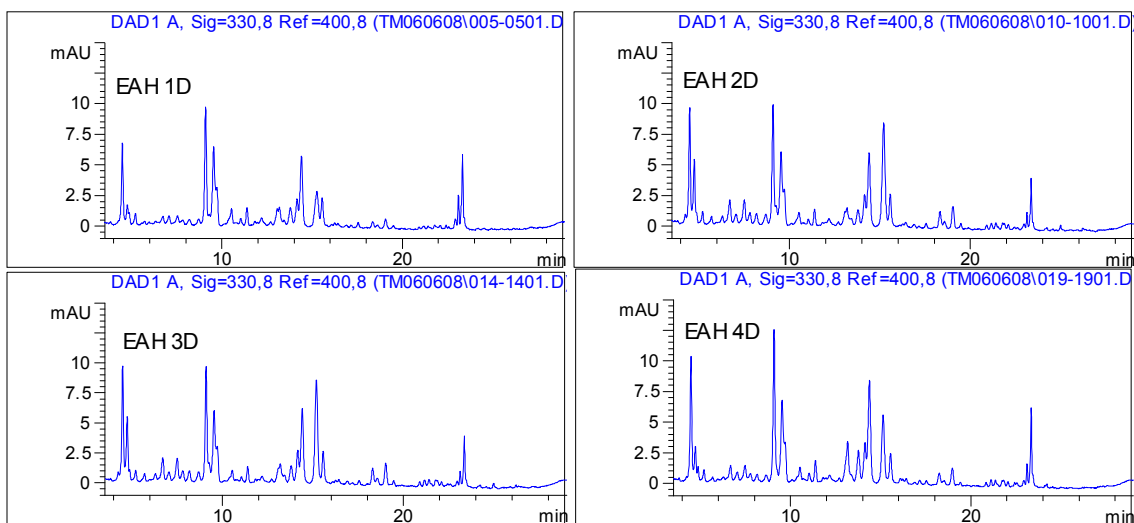


Figure 2. HPLC profiles of methanol extracts. The extraction methods used were, EAH1D, soxhlet-sequential; EAH2D, soxhlet-parallel; EAH3D, vortex-sonication; EAH4D, ASE.

In Figure 3, the chromatograms of ASE extracts with methanol, DEW (DMSO/ROH/water) and water extracts are compared.

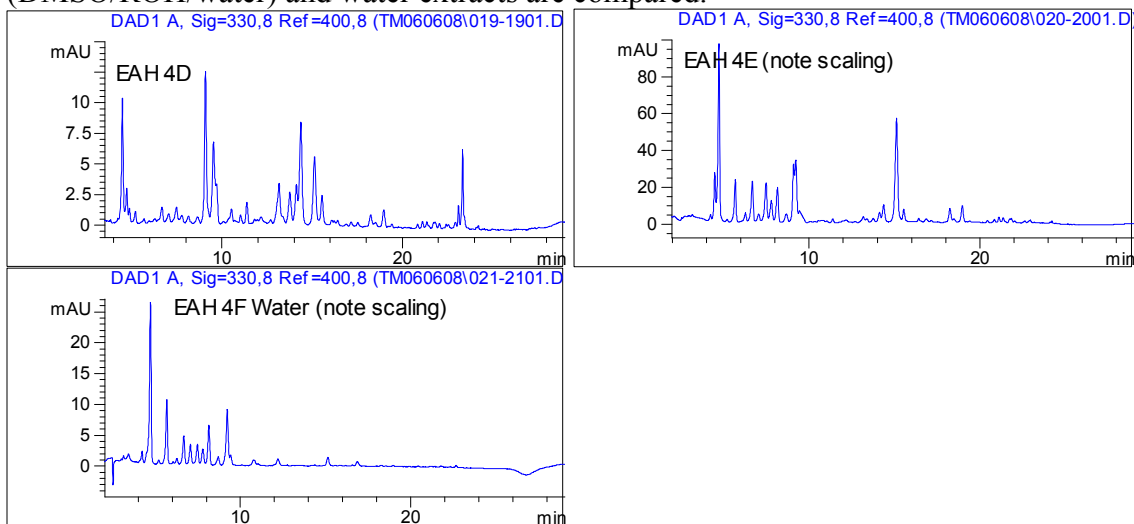


Figure 3. HPLC profiles of ASE extracts using methanol, EAH4D; DMSO/ROH/Water, EAH4E; and water, EAH4F.

Discussion and Conclusion

From the results, both yields and chromatogram profiles of the extracts from the same extraction solvent are fairly close, in spite of the extraction methods. Thus it can be concluded that the extraction techniques are not essential to the chemical extractability or the quality of the extract. The solvents used in the process have far more critical roles on the two extract qualities.

The selection of an extraction technique will be depending on operational requirements. These factors include, but not limited to, instrument cost, labor cost, operational cost, consumable cost. The extraction time is another factor that directly related to the throughput of the method, which is a key consideration in certain laboratory practice. In table 2, some non-technical factors are compared for ASE, SAE and soxhlet.

Table 2 Comparison of several factors that affects the performance of the technologies.

| Items\Technologies | ASE | SAE | Soxhlet-parallel |
|---------------------|-----------------|-------|------------------|
| Extraction time | short | long | long |
| Operation Labor | low (automated) | mid | mid |
| Operational Cost | mid | low | high |
| Instrument Cost, \$ | 50,000 | 1,500 | 300 |

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