Comparison the Soxhlet and Supercritical Fluid Extraction of Nettle Root (*Urtica dioica* L.)

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Abstract

The leaves of stinging nettle (Urtica dioica L.) are used for medical purposes since centuries while the nettle roots are applied only in the last decades. According to the latest researches the root of nettle contains many important ingredients, for example scopoletin, sterols, fatty acids, polysaccharides, polyphenols, minerals, etc. The polyphenols have antioxidant effect, reduce the hematological disorders, but also have anti-cancer and anti-bacteriological effects. The H-donating ability expresses the antioxidant activity.

In this study two kinds of extraction techniques and three kinds of solvents have been applied. The extraction techniques were: Soxhlet extraction with 96% ethanol, Soxhlet extraction with n-hexane and supercritical fluid extraction (SFE) with CO_2. All the measurements and the analysises were repeated three times.

Comparing the yields of the three extraction methods it was established that the highest yield value was reached by Soxhlet extraction with ethanol. The Soxhlet extraction and the supercritical extraction has lower yield, the total polyphenol content and the H-donating ability of these extracts were low.

Keywords

Soxhlet extraction, supercritical fluid extraction, Stinging nettle roots, Total polyphenol content, H-donating ability

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1 Introduction

Medical plants are largely investigated nowadays, because they are natural products with biologically active organic compounds, which usually are not aggressive, and do not cause severe side effects. The medical herbs are applied in the folk therapy since centuries [1,2].

The stinging nettle (*Urtica dioica* L.) is a well-known herb, it can be found everywhere in Asia, Europe and in Hungary, as well. The nettle leafs are used since ancient times [3]. The most important effects of nettle are anti-cancer, anti-inflammatory, hematological impacts [4], antibacterial effect [5], they are used for treatment of diabetes [2], cardiovascular deceases [4], rheumatic and allergic conditions [3], etc.

In contrast with the widespread investigation of nettle leafs and flowers, the nettle roots are discovered only in the last decades [6,7].

The aim of this study was on one hand to investigate the total polyphenol content of the nettle root extract, because the polyphenols are main ingredients of the nettle [8]. On the other hand to determine the H-donating ability, it is the main indicator of the antioxidant ability of the material.

The measurement results of the three types extraction were compared: Soxhlet extraction with 96% ethanol, Soxhlet extraction with n-hexane and supercritical fluid extraction (SFE) with CO_2.

2 Materials and methods
2.1 Row material

Stinging nettle (*Urtica dioica* L.) roots were applied to gain valuable components. The roots were collected by the Fitodry Ltd at south of Hungary, than dried naturally.

The airborn dried roots were chopped into 1 mm size by Fritsch mill, than the characteristic particle size of the chopped nettle root and the uniformity factor of the conglomerate was determined with sieve analysis, using Retsch AS 200 device. The pore size of the vibrated sieves was 0, 0.1, 0.25, 0.315, 0.4, 0.5, 0.63, 0.8 and 1.0 mm. The retained material on each sieve was measured by tare balance (Fig.1).

Fig. 1 Sieve shaker

Fig. 2 Soxhlet extractor

Before the extraction measurements the dry matter of the naturally dried root pieces was determined in an owen at 104 °C. The initial dry content of the input material was 91.07% w/w.

In the extractions naturally dried and chopped stinging nettle roots were applied. Different extraction methods: Soxhlet and supercritical fluid extraction (SFE) and different solvents (ethanol, n-hexane, supercritical CO_2) were applied.

2.2 Extraction types

In a *Soxhlet extractor* (Fig. 2) normally a solid material containing some of the desired compounds is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the extractor. The extraction solvent to be used is taken into a boiling distillation flask.

The solvent is heated to gain reflux. The chamber containing the solid material is slowly filled with hot solvent. Some of the desired compound will then dissolve in the hot solvent. When the Soxhlet chamber is almost full, the chamber is automatically empty by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.
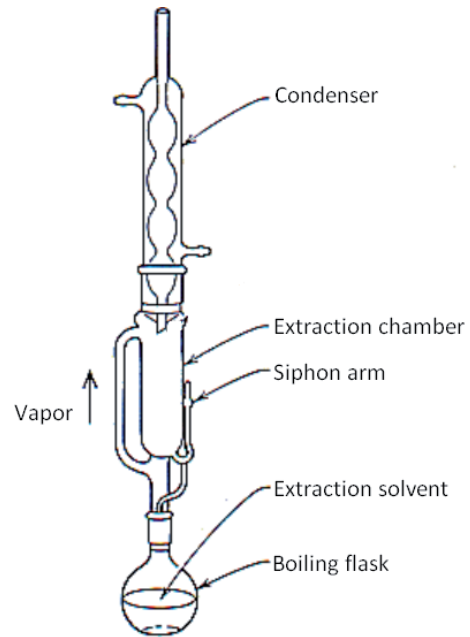
In our case the extraction time was 14-15 hour, in each hour with 4 cycles. During each cycle, a portion of the soluble compounds were dissolved in the solvent. After many cycles the desired compounds were concentrated in the distillation flask.

Supercritical fluid extraction (SFE) is applied for herbs' separation since about 10-15 years, because it is suitable for careful, low temperature extraction of herbs' active ingredients.

As a solvent normally carbon dioxide is used, because it is cheap, not toxic, its critical temperature is very low (so substantial heat damage can be avoided), and at the end of the procedure it can be perfectly separated from the dissolved ingredients. The diffusivity of the supercritical fluid (SCF) is 100 times more than liquid diffusivity, and 1,000 - 10,000 times less than that of gases. Viscosity for SCF is similar with gases and 10 times less than that of liquids.
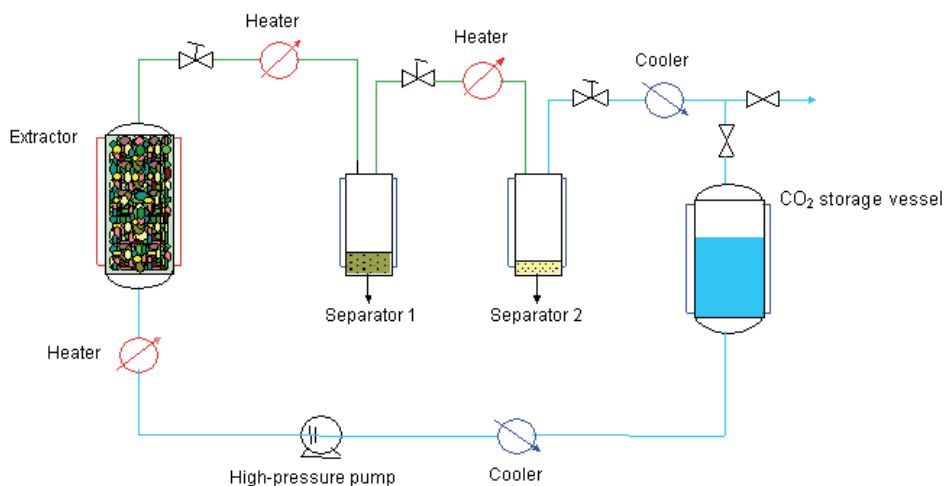

Fig. 3 Flowchart of supercritical fluid extraction (SFE) equipment

The flowchart of the pilot SFE is shown in Fig. 3. The chopped root is placed into the extraction chamber, which can contain 1 kg material. The mobile phase, the liquid CO_2 is pressed to the extractor with a high pressure pump. The solving power of SCF can be promoted by tuning temperature and pressure.

The basic principle of SFE is that in the extractor the feed material is contacted with the supercritical fluid, and soluble substances from the feed solve into the supercritical phase. In our case 300 bar pressure and 40 °C temperature was applied in the extractor. After the extraction the supercritical fluid, containing the dissolved substances, flows through a pressure reducing valve reaching 40 bar and 20 °C in the first separator. The extract remains in separator 1, while the CO_2 passes through separator 2 and leaves the system. The stinging nettle does not contain volatile oil, therefore separator 2 was not used. The dissolution rate decreases in function of time, the extraction is less and less economic, after some hours – in our case after 3.5 h – the extraction has to be stopped.

The SCF may be recompressed to the extraction conditions and recycled.

2.3 Analysis

The total polyphenol content and the H-donating ability were determined by the literature [9,10]. The extracton yield was measured by tare balance.

2.4 Total polyphenol content

The total polyphenol content in the extracts was determined according to the literature [9,10], by Campsec M501 spectrophotometer at 760 nm, after half an hour incubation at room temperature. The reagent was the Folin-Ciocalteau solution, the reference was pyrogallol solution, identical background was distilled water. The concentration of the extract solution was 2.5 mg/mL 96% ethanol. From this sample 0.8 mL was mixed with 4 mL distilled water, 0.4 mL Folin-Ciocalteau reagent and 14.8 mL Na_2CO_3 (29 g/L).

2.5 H-donating ability

The H-donating ability was determined by the method of Hatano et al. with some modification. [9]. 0.01 gram sample from the three different extracts was dissolved in 20 mL methanol. From each methanol solution 0.1, 0.2, 0.5, 0.75 and 1.0 mL were diluted with 2.5 mL DPPH solution. Before the measurement the absorbance of the DPPH solution was adjusted to 0.7-0.9. The control solution was methanol.

The absorbance of the solutions was measured after 30 minute incubation at 517 nm, by Camspec M501 spectrophotometer. The Inhibiting% was calculated with the latter formula:

$$Inhibiting\% = 100*(A_{control} - A_{sample}) / A_{control}$$

where $A_{control}$ is the absorbance of the DPPH solvent, A_{sample} is the absorbance of the sample.

The inhibition value of the different samples was compared at 30%, which is that concentration value, when the inhibition reaches the 30%.

3 Results and discussion
3.1 Results of sieve analysis

Table 1 contains measured points. Fig. 4 shows the average points of three parallel measurements.

The characteristic particle size and the uniformity factor were calculated by the Statistica program, using the RRB-expression [11]. The average particle size was $x_o = 0.505$ mm, the uniformity factor was n = 2.00.

The particle size distribution has some higher values below 0.15 mm. It means the structure of nettle root is not homogenous, therefore lower (~0.15 mm) diameter particles appear in the chopped nettle root.

3.2 Results of Soxhlet extraction yields

Soxhlet extractions were performed with naturally dried nettle root. Experimental data obtained by ethanol are collected in Table 2. The yield was defined as the dry matter content of the extract (DME) related to the initial dry matter content (DMC).

Table 1 Data of sieving

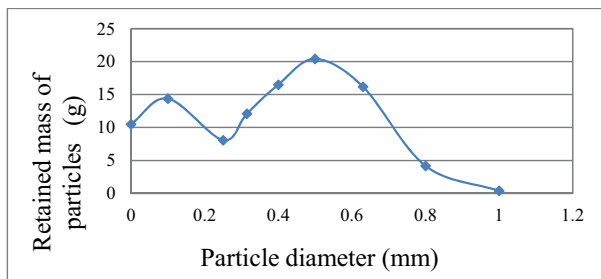| Diameter of mesh sieve	Mass of sieves	Mass of sieves + nettle root	Mass of nettle root	Residual in percent %
0	362.9	373.4	10.5	10.50
0.1	248.9	263.3	14.4	14.40
0.25	279.5	287.6	8.1	8.10
0.315	295.9	308	12.1	12.10
0.4	366.4	382.9	16.5	16.50
0.5	306.5	326.9	20.4	20.40
0.63	381.9	398.1	16.2	16.20
0.8	399.6	403.8	4.2	4.20
1	409.6	410	0.4	0.40

Fig. 4 Particle size distribution of chopped nettle root.

The average value of the yield was 14.14% in case of ethanol.

Table 3 shows the results with n-hexane as a solvent.

The n-hexane produced only 0.768% yield, which is much less, than that of the yield with ethanol.

3.3 Results of supercritical extraction yields

The supercritical fluid extraction experiments applied the same dried nettle roots as row material, and supercritical CO_2 as solvent. The pressure in the extractor was 298-302 bar, the mass flow rate of CO_2 was ≈ 7 kg/h. The pressure in the extractor varied less than 1%, because of the instability of the pressure regulation.

Table 4 contains experimental data. As an example the steps of the SFE extraction at ~ 300 bar are detailed in this Table. The

samples were taken every ~ 35 min. After each time section the dry matter content of the extract was measured in gram and calculated in %. The used CO_2 was noted as well. The mCO_2 is the mass of the applied CO_2 in one measurement step. The amount of the extract decreased in each time section, while the applied CO_2 increased.

The input quantity of nettle root was 1.0034 kg, and the dry matter content of it was 0.914 kg. The extract was collected in separator 1, its value was expressed in kg, and in percent related to the initial dried root.

Fig. 5 presents the yield of SFE extract in percent as a function of the applied CO_2, at 300 bar pressure.
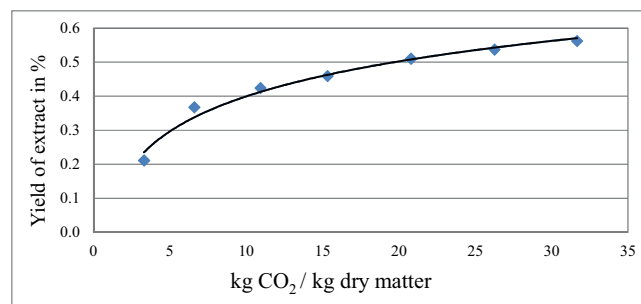

Fig. 5 Yield of SFE extracts in function of applied CO_2 at 300 bar pressure

Table 2 Soxhlet extraction data of dried nettle roots using ethanol 96%

Weight of dried nettle root	Dry matter content of dried nettle root DMC	Dry matter content of extract DME	(DME/DMC)*100 yield	(DME/DMC)*100 average yield
gram	gram	gram	%	%
19.81	18.16	2.53	13.90	
20.13	18.46	2.65	14.38	14.14

Table 3 Soxhlet extraction data of dried nettle roots using n-hexane

Weight of dried nettle root	Dry matter content of dried nettle root DMC	Dry matter content of extract DME	(DME/DMC)*100 yield	(DME/DMC)*100 average yield
gram	gram	gram	%	%
22.4170	20.44	0.149	0.728	
20.6072	18.77	0.152	0.808	0.768

Table 4 Supercritical extraction steps

Measurements	Time sections min	mCO_2 kg	Dry matter content of extract			Used CO_2 kg CO_2/kg dry matter
			kg	%	$\sum$%	
1.	26	3.028	$1.93{*}10^{-3}$	0.211	**0.211**	**3.313**
2.	26	3.004	$1.43{*}10^{-3}$	0.156	**0.367**	**6.600**
3.	34	3.962	$0.52{*}10^{-3}$	0.057	**0.424**	**10.934**
4.	34	4.015	$0.32{*}10^{-3}$	0.035	**0.459**	**15.327**
5.	43	4.990	$0.47{*}10^{-3}$	0.051	**0.510**	**20.787**
6.	43	5.016	$0.25{*}10^{-3}$	0.027	**0.537**	**26.265**
sum	206	24.015	$4.92{*}10^{-3}$	0.537		31.663
Total input quantity of dried roots: 0.914 kg						

The yields of the above mentioned SFE extraction steps at 300 bar are shown in Fig.5. The samples were taken every ~ 35 min. In function of time the extraction is less and less economic, because the increase of yield slows down.

3.4 Comparison of polyphenol contents and H-donating abilities of the three types of extracts

To compare the quality of the three types of extracts, total polyphenol content and H-donating ability were determined.

In Fig. 6 the polyphenol content is shown, expressed in pyrogallol equivalent %. As it can be seen, much more polyphenols were detected with Soxlet extraction using ethanol, than with Soxhlet using n-hexane, and more than in the SFE.
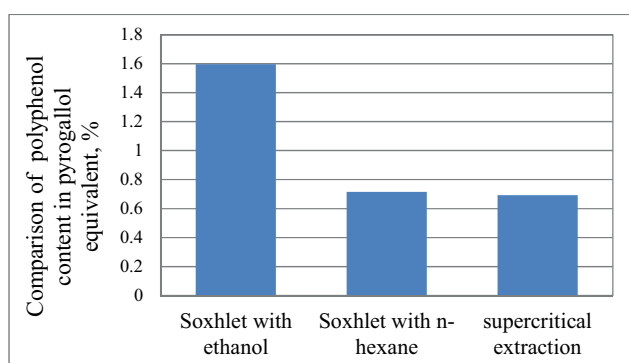
Fig. 6 Comparison of polyphenol content expressed in pyrogallol, mg/mL

The H-donating ability was compared at 30% inhibition value (Fig.7). If the concentration of the extract is low - the 30% concentration was reached at low extract value - than the H-donating ability is high.
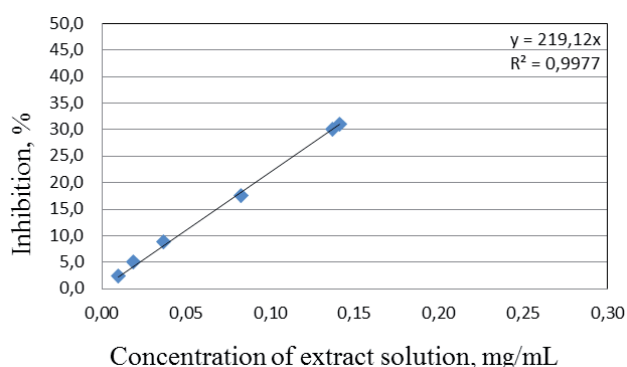
Fig. 7 H-donating ability of the extract, SFE 300 bar

Figure 8 presents the H-donating ability of the three extracts. In case of Soxhlet extraction with 96% ethanol, the best H-donating ability was measured.

The Soxhlet extraction with n-hexane and the supercritical extraction produced lower H-donating ability than the Soxhlet extraction with ethanol.
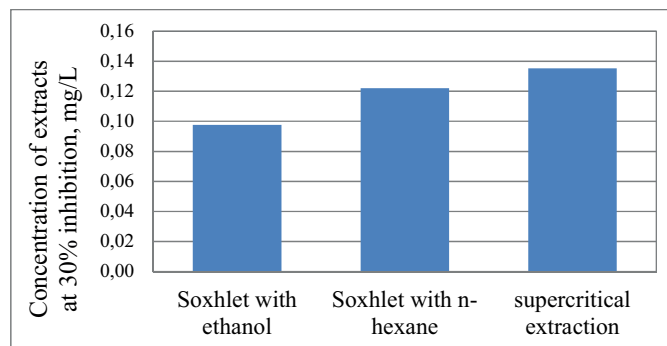
Fig. 8 Concentration of extract at 30% inhibition, mg/mL

4 Conclusion

Extraction of nettle root was investigated by different methods and solvents. Comparing the experimental results the next attributes were established:

In case of Soxhlet extraction with 96% ethanol the polyphenol content, expressed in pyrogallol equivalent, was 1.6%, while the above mentioned value in case of Soxhlet extraction with n-hexane and supercritical fluid extraction was only the half of it, in both case about 0.7%. It seems, the ethanol is the best solvent in our case to reach the highest polyphenol yield.

The H-donating abilities of the extracts of stinging nettle root were low in all the three extraction types, the concentration at 30% inhibition changed between 0.095 – 0.135 mg/L. If the H-donating ability is high, than the material is proper for the reduction of free radicals in the human body. The highest concentration of extract - means the lowest DPPH value - was achieved using SFE. It suggests that this extract practically does not have inhibition effect, or contributes to the formation of free radicals. The understanding of this unusual fact will be the aim of the next experiments.

The particle size distribution shows, the nettle root is not a homogeneous material.

To sum the results: The SFE is not a proper method for the removal of polyphenols and to produce high H-donating ability, some other constituents of the stinging nettle root have to be tested. The extract of the Soxhlet extraction with ethanol or n-hexane contains the unpleasant solvents, which have to be separated, while the carbon dioxide from the SFE extract can be removed easily.

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