Determination of phenolic compounds in above- and underground organs of dropwort (*Filipendula vulgaris* Moench)

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INTRODUCTION

Dropwort (*Filipendula vulgaris* Moench, *Rosaceae)* is a perennial naturally occurring on sunny, semi-dry, limestone meadows and neglected areas in Europe and Asia. All organs of this plant – rhizomes, tuberous roots, leaves and flowers are a rich source of phenolic compounds, especially flavan-3-ols and phenolic acids. Raw materials – herb (*Filipendulae vulgaris herba*) and underground parts (*Filipendulae vulgaris radix*) have been used in traditional European medicine as anti-inflammatory, antipyretic, analgetic, antirheumatic, diuretic, and diaphoretic agents. The decoction of underground organs has often been used to treat kidney problems, breathlessness, wheezing, sore throat, congestion, stomachache, and diarrhea. Moreover, the tuberous roots and young leaves are edible – cooked as a vegetable or eaten raw as a component of salads. Leaves and flowers are decorative and can be used for beddind and cut flowers.

The aim of this study was to find the optimum extraction conditions (method and solvent) for determination of phenolic compounds in rhizomes (r), tuberous roots (t), leaves (l), and flowers (f) of dropwort.

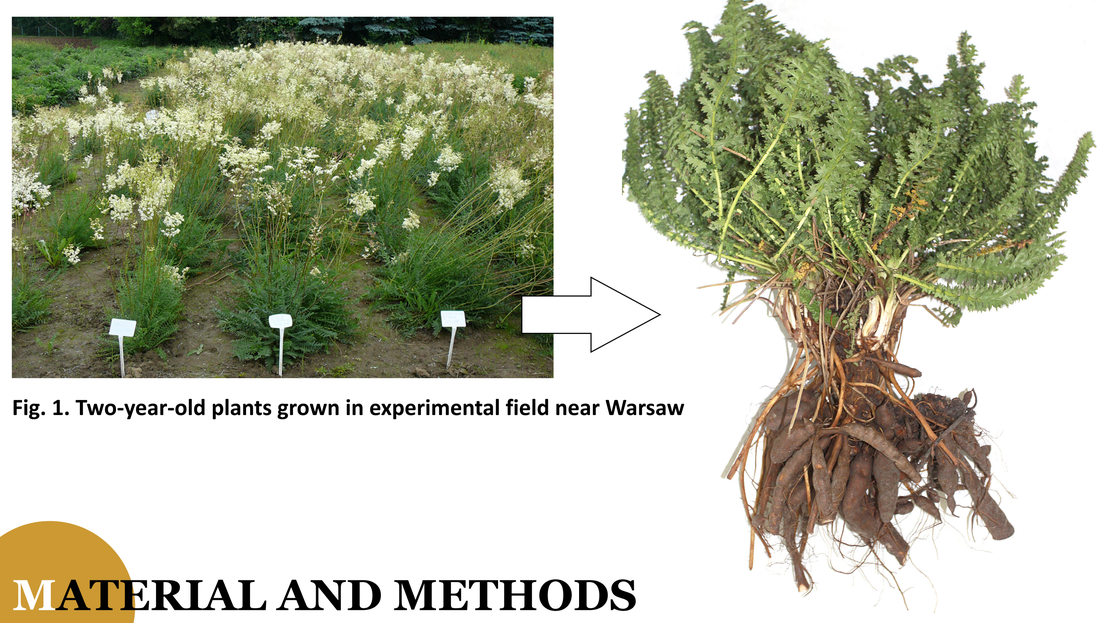

Fig. 1. Two-year-old plants grown in experimental field near Warsaw

MATERIAL AND METHODS

The plant material was harvested from the plantation of dropwort established in the experimental field of the Department of Vegetable and Medicinal Plants, WULS – SGGW (Fig. 1) from seeds collected from natural sites located in Podlasie region, Poland. Two-year-old plants were dug out in October. Raw material was dried by convection at 60 °C and then ground in a laboratory mill (Fig 2.).

Fig. 2. a) rhizomes (r) b) tuberous roots (t) c) leaves and flowers (l) d) flowers (f)

Homogenized, air-dry raw material (1 g) was extracted using two periodic extraction methods – under reflux (traditional way of extraction for this raw material) (A) and sonication-assisted solvent extraction (B), as well as two continuous extraction methods – in classic Soxhlet apparatus (C) and in modified, automated Soxhlet apparatus (Büchi Extraction System B-811 – hot extraction and solvent evaporation, Fig. 3) (D). Ethanol 40% as well as methanol were applied as extraction medium.

After evaporation of solvent, the residue was dissolved in 10 ml of proper solvent. The obtained extracts were filtered with Supelco Iso-Disc™ Syringe Tip Filter Unit, PTFE membrane, diameter 25 mm, pore size 0.20 µm and subjected to HPLC. The analyses were performed using a Shimadzu HPLC system equipped with photodiode array detector SPD-M10A VP PDA, autosampler SIL-20 and Class VP 7.3 chromatography software. Separation was obtained by 2.6 µm C18 reversed-phase column with core-shell technology. Binary gradient of mobile phase A (deionised water/phosphoric acid 0.1%) and B (ACN/phosphoric acid 0.1%) was used. The following conditions were applied: flow rate 1.0 ml×min^{-1}, oven temperature 31 °C, total time of analysis 15 min, injection volume: 1 µl. UV-spectra were recorded from 190 to 450 nm. Peak identification was confirmed by comparison of retention time and spectral data with adequate parameters of standards purchased from ChromaDex. For quantitation of investigated compounds the five-point calibration curve method was used in CLASS VP 7.3 chromatography software. The content of the determined compounds was calculated in mg×100g^{-1} dry matter.

The results were analysed with one-way ANOVA and Tukey's HSD test at α=0.95 using Statgraphics Plus for Windows v. 4.1 software.

RESULTS

Table 1. Comparison of extraction parameters

Extraction method	Extraction time	Solvent usage	Extraction cycles	Solvent evaporation
A) Under reflux	1.5 – 2 h	100 ml	2	manual in external evaporator
B) Sonication-assisted extraction	1 – 1.5 h	100 ml	2	manual in external evaporator
C) Classic Soxhlet apparatus	50 h	250 ml	approx. 10	manual in external evaporator
D) Büchi Extraction System B-811	4 – 6 h	100 ml	20	automated

Fig. 3. Büchi Extraction System B-811

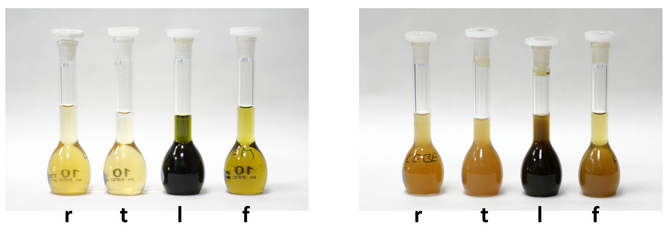

Fig. 4. Extracts obtained in Büchi Extraction System with methanol (left) and ethanol 40% (right)

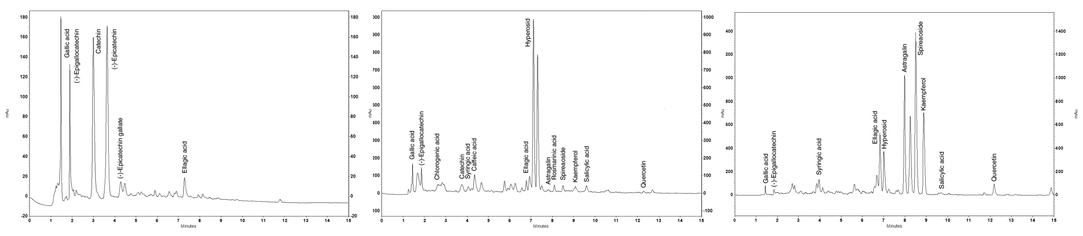

Fig. 5. Sample chromatograms of the extracts obtained in Büchi Extraction System from tuberous roots (left), leaves (center) and flowers (right) (Spectrum Max Plot)

Table 2. Content of phenolic compounds - rhizomes (mg 100g^{-1} dry weight)

Catechin derivatives	Solvent	Extraction Method A	B	C	D	Mean for solvents
(-)-Epigallocatechin	Ethanol	391.53	400.52	240.40	230.52	315.74 a
	Methanol	252.70	240.42	320.28	337.54	287.74 b
	Mean for methods	322.11 a	320.47 a	280.34 b	284.03 b	
(+)-Catechin	Ethanol	491.36	503.00	528.04	542.50	516.22 b
	Methanol	517.87	458.60	567.80	549.06	523.33 a
	Mean for methods	504.62 b	480.80 c	547.92 a	545.78 a	
(-)-Epicatechin	Ethanol	367.02	384.05	437.33	428.20	404.15 b
	Methanol	402.94	341.75	448.83	459.68	413.30 a
	Mean for methods	384.98 b	362.90 c	443.08 a	443.94 a	
(-)-Epigallocatechin galate	Ethanol	148.72	150.42	147.88	145.13	148.04 b
	Methanol	157.44	151.71	157.80	160.86	156.95 a
	Mean for methods	153.08 n.s.	151.06 n.s.	152.84 n.s.	152.99 n.s.	

Phenolic acids	Solvent	Extraction Method A	B	C	D	Mean for solvents
Ellagic acid	Ethanol	35.58	33.57	33.89	35.86	34.72 a
	Methanol	20.80	18.85	17.10	16.71	18.36 b
	Mean for methods	28.19 a	26.21 b	25.49 b	26.28 b	
Gallic acid	Ethanol	192.33	202.82	211.88	220.85	206.97 a
	Methanol	99.97	118.98	105.80	110.16	108.73 b
	Mean for methods	146.15 b	160.90 a	158.84 a	165.51 a	

Table 3. Content of phenolic compounds - tuberous roots (mg 100g^{-1} dry weight)

Catechin derivatives	Solvent	Extraction Method A	B	C	D	Mean for solvents
(-)-Epigallocatechin	Ethanol	319.33	331.56	291.00	297.62	309.88 a
	Methanol	255.09	228.74	254.85	254.58	248.31 b
	Mean for methods	287.21 a	280.15 ab	272.92 b	276.10 b	
(+)-Catechin	Ethanol	399.26	402.54	641.38	634.75	519.48 b
	Methanol	605.50	512.18	646.13	651.99	603.95 a
	Mean for methods	502.38 b	457.36 c	643.75 a	643.37 a	
(-)-Epicatechin	Ethanol	413.45	401.35	659.79	698.44	543.25 b
	Methanol	676.75	641.95	765.56	793.81	719.51 a
	Mean for methods	545.10 c	521.65 c	712.67 b	746.12 a	
(-)-Epigallocatechin galate	Ethanol	364.57	315.37	447.29	454.08	395.33 b
	Methanol	507.41	349.86	500.71	520.34	469.58 a
	Mean for methods	435.99 c	332.61 d	474.00 b	487.21 a	

Phenolic acids	Solvent	Extraction Method A	B	C	D	Mean for solvents
Ellagic acid	Ethanol	77.62	34.57	122.04	130.90	91.28 a
	Methanol	57.46	26.24	74.83	78.12	59.16 b
	Mean for methods	67.54 b	30.40 c	98.44 a	104.51 a	
Gallic acid	Ethanol	625.77	682.00	743.68	761.72	703.29 n.s.
	Methanol	658.38	596.53	777.96	790.87	705.93 n.s.
	Mean for methods	642.07 b	639.27 b	760.82 a	776.29 a	

Table 4. Content of phenolic compounds - leaves (mg 100g^{-1} dry weight)

Catechin derivatives	Solvent	Extraction Method A	B	C	D	Mean for solvents
(-)-Epigallocatechin	Ethanol	167.83	131.95	216.38	229.04	186.30 a
	Methanol	130.94	88.68	101.54	117.46	109.65 b
	Mean for methods	149.38 b	110.31 c	158.96 b	173.25 a	
(+)-Catechin	Ethanol	541.23	558.00	661.90	670.05	607.79 a
	Methanol	574.96	523.89	527.40	546.73	543.24 b
	Mean for methods	558.09 c	540.95 d	594.65 b	608.39 a	

Flavonoid	Solvent	Extraction method A	B	C	D	Mean for solvents
Hyperoside	Ethanol	270.58	280.64	386.41	392.06	332.42 a
	Methanol	325.88	237.93	328.45	314.76	301.75 b
	Mean for methods	298.23 b	259.28 c	357.43 a	353.41 a	
Astragalin	Ethanol	222.33	194.73	155.40	170.59	185.76 a
	Methanol	164.87	170.01	156.41	169.13	165.11 b
	Mean for methods	193.60 a	182.37 b	155.91 d	169.86 c	
Spiraeoside	Ethanol	77.44	77.72	75.69	72.98	75.96 a
	Methanol	76.55	66.08	75.13	73.60	72.84 b
	Mean for methods	76.99 a	71.90 b	75.41 ab	73.29 ab	

Phenolic acids	Solvent	Extraction Method A	B	C	D	Mean for solvents
Ellagic acid	Ethanol	49.48	37.38	110.82	127.42	81.28 a
	Methanol	34.19	15.46	44.90	34.22	32.19 b
	Mean for methods	41.84 b	26.42 c	77.86 a	80.82 a	
Gallic acid	Ethanol	431.68	633.4	780.53	812.65	664.56 a
	Methanol	169.81	124.00	163.58	173.99	157.84 b
	Mean for methods	300.74 d	378.70 c	472.06 b	493.32 a	
Salicylic acid	Ethanol	12.66	9.86	11.92	11.07	11.38 a
	Methanol	8.83	6.86	9.78	9.39	8.72 b
	Mean for methods	10.74 ab	8.36 c	10.85 a	10.23 b	
Chlorogenic acid	Ethanol	127.84	267.08	531.38	539.27	366.39 a
	Methanol	333.07	234.49	262.56	284.02	278.53 b
	Mean for methods	230.45 d	250.78 c	396.97 b	411.64 a	
Caffeic acid	Ethanol	55.51	82.36	99.33	111.34	87.13 a
	Methanol	90.65	65.35	85.08	87.84	82.23 b
	Mean for methods	73.08 c	73.86 c	92.20 b	99.59 a	
Rosmarinic acid	Ethanol	48.33	47.82	66.20	66.58	57.23 b
	Methanol	65.18	52.18	55.42	64.31	59.27 a
	Mean for methods	56.75 d	50.00 c	60.81 b	65.44 a	

Table 5. Content of phenolic compounds - fowers (mg 100g^{-1} dry weight)

Phenolic acids	Solvent	Extraction Method A	B	C	D	Mean for solvents
Syringic acid	Ethanol	233.79	193.88	175.88	188.86	198.10 b
	Methanol	224.81	175.74	200.16	205.25	201.49 a
	Mean for methods	229.30 a	184.81 d	188.02 c	197.05 b	
Gallic acid	Ethanol	551.02	428.72	501.89	519.56	500.30 b
	Methanol	629.61	331.29	534.52	558.75	513.54 a
	Mean for methods	590.31 a	380.00 d	518.20 c	539.16 b	

Flavonoid	Solvent	Extraction method A	B	C	D	Mean for solvents
Hyperoside	Ethanol	347.94	297.03	353.55	356.19	338.67 a
	Methanol	322.97	269.41	359.08	377.85	332.33 b
	Mean for methods	335.45 c	283.22 d	356.31 b	367.02 a	
Astragalin	Ethanol	484.13	417.51	563.54	595.87	515.26 b
	Methanol	537.27	451.26	658.12	714.92	590.39 a
	Mean for methods	510.70 c	434.38 d	610.83 b	655.40 a	
Spiraeoside	Ethanol	539.56	454.04	685.62	703.30	595.63 b
	Methanol	809.77	469.21	867.56	910.78	764.33 a
	Mean for methods	674.66 c	461.63 d	776.59 b	807.04 a	
Kaempferol	Ethanol	243.67	219.44	299.54	298.39	265.26 b
	Methanol	299.95	225.97	321.04	324.72	292.92 a
	Mean for methods	271.81 b	222.70 c	310.29 a	311.55 a	

A - Under reflux, B - Sonication-assisted solvent extraction, C - Classic Soxhlet, D - Büchi Extraction System

Values marked with the same small letters do not differ significantly at α=0.95, Tukey's HSD test, n=3

CONCLUSIONS

1. **There were no significant differences in the content of examined compounds between extracts obtained with modified and classic Soxhlet, but the extraction was 12.5 times shorter (4 h) and the solvent consumption was 2.5 times lower (100 ml). Moreover, the modified Soxhlet apparatus allows for the automated evaporation of the solvent. In case of all other investigated methods, the extraction solvent must be removed with additional evaporator.**
2. **Sonication-assisted solvent extraction was the shortest investigated method (1 h including solvent evaporation), but the content of phenolic compounds in obtained extracts was lower in comparison with Soxhlet extraction.**
3. **The content of investigated phenolic compounds in extracts obtained with 40% ethanol and methanol was comparable.**

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