

THE INFLUENCE OF ULTRASOUND ASSISTED EXTRACTION ON THE ISOLATION OF BIOACTIVE COMPOUNDS FROM NETTLE LEAVES



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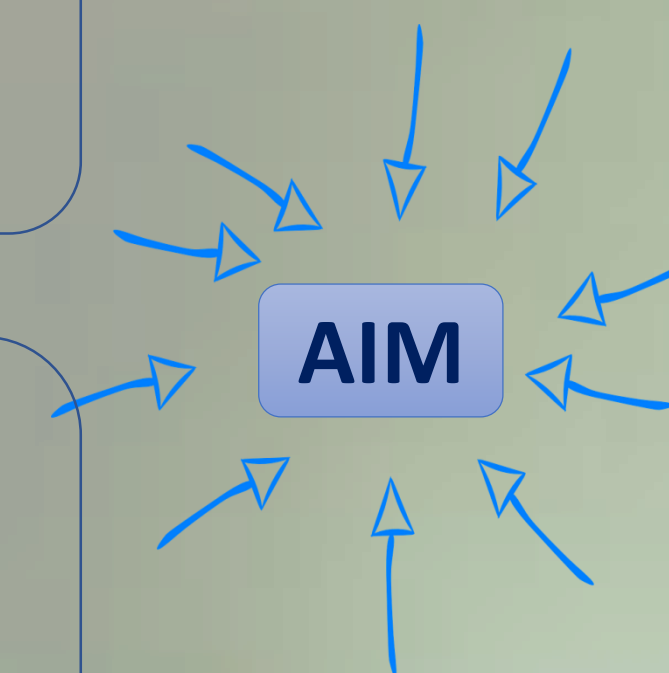
INTRODUCTION

Urtica dioica L., commonly known as nettle, is a perennial plant from the *Urticaceae* family. Nettle leaves are rich in bioactive compounds, such as the phenolic compounds, which are exceptionally important for the growth and reproduction of the plant and are considered to have a significant effect on human health and are strong antioxidants (Adhikari et al., 2016). For the isolation of the phenolic compounds, various extraction techniques are used, such as conventional methods requiring extended extractability time and a higher amount of solvent. One of the most commonly used non thermal eco-friendly techniques for isolation of bioactive compounds from plants is ultrasound assisted extraction (UAE), whose advantages are shortening the time of extraction, reduced solvent consumption, reduced extraction temperature, and lower energy consumption (Wang & Weller, 2006).



The aim of this study was to determine the optimal conditions for efficiency of phenolic compounds isolation using ultrasound assisted extraction from nettle leaves.

The extraction was performed at constant frequency (24 kHz) and the 2 mm probe. Sources of variation were: the amplitude (25, 50, 75 and 100 %) and the extraction time (5, 10, 15, 20, 30 and 40 minutes).



MATERIALS AND METHODS

NETTLE LEAVES



Sample of lyophilized nettle leaves (*Urtica dioica* L.) was used during the research. Fresh leaves were collected from Žumberak area, during the spring of 2019. After the lyophilization (24h, -50°C) nettle leaves were grounded into fine powder using mortar and pestle which were used for the ultrasound assisted extraction.

ULTRASOUND ASSISTED EXTRACTION



- UP200Ht (200 W, 24kHz)
- Hielscher GMBH (Teltow, Germany)
- Sonotrode: 2 mm
- Solvent: 96% EtOH
- Amplitude: 25, 50, 75 and 100 %
- Extraction time: 5, 10, 15, 20, 30 and 40 min

SPECTROPHOTOMETRY

STATISTICAL ANALYSIS

Total phenolic content (TPC)

- Folin Ciocalteu method
- 765 nm
- Shortle et al., 2014

Results were statistically analyzed using multivariate analysis of variance (MANOVA) and Tukey's HSD test at significance level $p \leq 0,05$



CONCLUSIONS

- UAE has been proven effective for extraction of phenolic compounds obtained from nettle leaves
- MANOVA results showed that the time of extraction significantly influenced the content of total phenols
- TPC was the highest at the time of extraction of 20 minutes. There was no significant variance between 20 and 40 minutes of extraction time
- Statistical analysis showed the highest TPC was at ultrasound amplitude of 75%
- When combining the time of the extraction and ultrasound amplitudes, MANOVA showed that the highest TPC was obtained at the 20 minutes of extraction time and ultrasound amplitude of 75%

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RESULTS AND DISCUSSION

Table 1. Influence of UAE time on content of TP (mg/100 g d.m.) in nettle leaves extracts

Source of variation	Total Phenols (mg/100 g d.m.)
Time (min)	$p \leq 0,05^*$
5	215,62±0,05 ^a
10	233,29±0,05 ^a
15	313,23±0,05 ^b
20	357,63±0,05 ^c
30	329,22±0,05 ^b
40	354,37±0,05 ^c

Extraction time of 5 min yielded the lowest TPC, indicating that 5 min is too short for more effective extraction. On the other hand, the highest TP yield was obtained when extraction time was 20 min, but there was not statistical difference ($p \geq 0,05$) between 20 min and 40 min.

Table 2. Influence of UAE amplitude on content of TP (mg/100 g d.m.) in nettle leaves extracts

Source of variation	Total Phenols (mg/100 g d.m.)
Amplitude (%)	$p \leq 0,05^*$
25	160,99±0,04 ^a
50	263,88±0,04 ^b
75	402,95±0,04 ^d
100	374,39±0,04 ^c

In many cases, lower amplitudes are not sufficient for the penetration of cell walls by ultrasound and the release of the phenolic compounds from the cell. On the other hand, the increase of the amplitudes can lead to degradation of phenolic compounds. Research results showed the highest TPC yield is given at 75%. The statistical analysis showed that there was a significant difference ($p \leq 0,05$) between 75% and 100%.

Table 3. Influence of UAE time and amplitude on content of TP (mg/100g d.m.) in nettle leaves

Source of variation		Total Phenols (mg/100 g d.m.)
Time (min)	Amplitude (%)	$p \leq 0,05^*$
5	25	163,46±0,10 ^a
5	50	160,00±0,10 ^a
5	75	281,00±0,10 ^{b,c}
5	100	258,00±0,10 ^b
10	25	164,54±0,10 ^a
10	50	171,03±0,10 ^a
10	75	344,30±0,10 ^{d,e}
10	100	253,27±0,10 ^b
15	25	177,24±0,10 ^a
15	50	235,21±0,10 ^b
15	75	400,45±0,10 ^{e,f}
15	100	440,00±0,10 ^g
20	25	165,00±0,10 ^a
20	50	271,09±0,10 ^b
20	75	563,11±0,10 ^b
20	100	431,31±0,10 ^{f,g}
30	25	164,48±0,10 ^a
30	50	412,00±0,10 ^f
30	75	353,03±0,10 ^{d,e}
30	100	387,37±0,10 ^{d,e,f}
40	25	131,27±0,10 ^a
40	50	334,00±0,10 ^{c,d}
40	75	475,78±0,10 ^g
40	100	476,42±0,10 ^g

When combining the time of the extraction and ultrasound amplitudes, MANOVA showed that 20 minutes of extraction time and ultrasound amplitude of 75% yielded the highest TP content

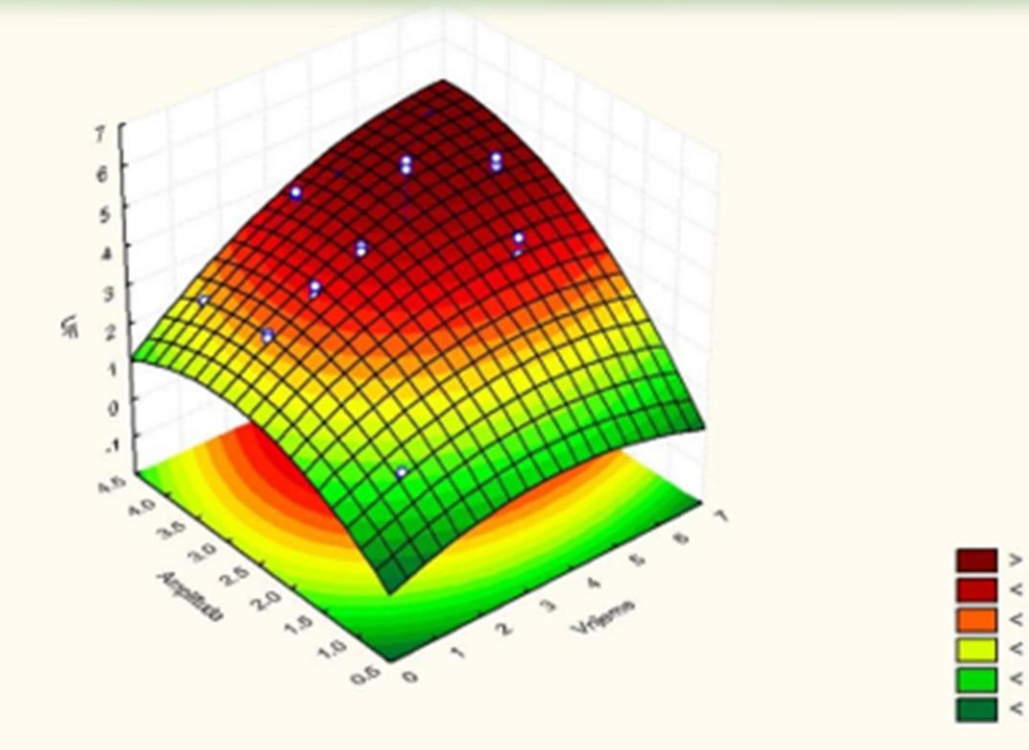


Figure 1. The response surface plot showing the effect of extraction time and amplitude on TPC in nettle leaves extracts



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