Ultrasonic-assisted Extraction of Arbutin from Mulberry Leaves

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Abstract

Arbutin is a bioactive compound found in mulberry leaves and has property as inhibitor of tyrosinase, which is the main enzyme in skin pigment synthesis. Arbutin is important in cosmetic industry. The objectives of this research was to investigate the extraction of arbutin from mulberry leaves by using ultrasonic-assisted extraction (UAE) to compare with the conventional extraction (CE). and to optimize the parameters included time period and ratio of dry weight of mulberry leaves to 70% ethanol in the design by Response Surface Methodology (RSM). Upon analysis of arbutin content by High performance Liquid Chromatography (HPLC), it was found that UAE provided arbutin at 4.5027 mg/g dry weight with 10 minutes of ultrasonication. While CE gave arbutin content of 1.8192 mg/g dry weight after soaking overnight. Both methods used 1:50 ratio of dry weight to solvent volume. Results demonstrated that UAE was an fast and suitable method for industrial applications.



Figure 1. Mullberry leaves

Objective

- To study the extraction of arbutin from mulberry leaves by UAE
- To compare the extraction efficiency of UAE with CE
- To investigate the effects of parameters, including ultrasonication period time (min) and ratio of dry weight of mulberry leaves to 70% ethanol, using Response Surface Methodology (RSM) as experimental design method

Introduction

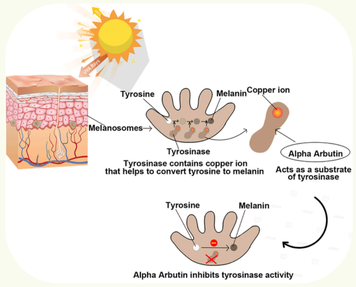


Figure 2. Representation mechanism of the action for skin lightening

Extraction Approach

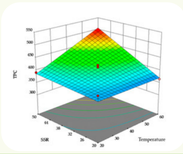
- Ultrasonic-assisted extraction (UAE)
- Conventional extraction (CE)



Optimization & Analysis



- Optimization using RSM



- Quantification by HPLC

Methodology

Preparation for extraction



fresh mulberry leaves were collected and cleaned



temperature: 50 °C
time: 96 hr.



the leaves were ground into a fine powder using a grinder

Conventional extraction (CE)



Mullberry leaf powder was mixed with 70% ethanol and soaked overnight at room tempe.





Water bath temperature : 40 °C
Rotation speed: 100 rpm
Evaporation: 15 min

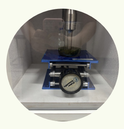


Drying time: 24 hr.

Ultrasonic-assisted extraction (UAE)



Mullberry leaf powder was mixed with 70% ethanol



Power 400 W
Amplitude: 55% Pulse
cycle: 0.7s





Water bath temperature : 40 °C
Rotation speed: 100 rpm
Evaporation: 15 min



Drying time: 24 hr.

HPLC Analysis

Preparation sample



Samplediluted with methanol



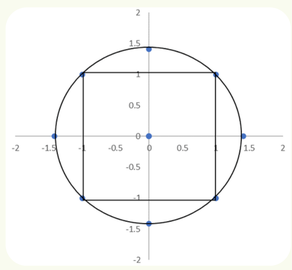
Membrane filter: 0.45 µm

HPLC Analysis



Condition Mobile phase: MeOH: H_2O : 0.1 M HCl (89 : 10 : 1, v/v)
Column: Reversed-phase C18
Flow rate: 1.0 ml/min Detection: UV Detection (240 nm) Injection Volume: 10 µL
Column temperature: 27 °C

Experimental Design



The plotted graph visually represents the experimental design. The square represents the factorial points, which show the combinations of high and low levels of the showing the variables. This design allows accurate modeling and optimization of extraction conditions.

Figure 6. Representation of the central Composite Design (CCD) plots in this research, resulting in a total of 11 experimental runs

Results and Discussion

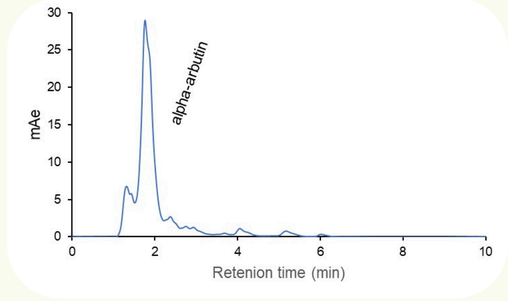


Figure 3. HPLC chromatogram of alpha-arbutin form CE showing the characteristicpeak at the retention time of approximately 1.8 minutes.

Table 1. Different extraction conditions for UAE1-UAE11 used in the ultrasonic-assisted extraction experiment.

UAE (No.)	Dry weight (g)	Time (min)	Volume of arbutin standard (mg/g)
1	1.3017	6.30	16.376
2	1.3009	13.30	19.971
3	2.7013	6.30	12.621
4	2.7016	13.30	13.958
5	1.0037	10.00	9.023
6	3.0067	10.00	17.828
7	2.0006	5.00	6.678
8	2.0027	15.00	11.451
9	2.0003	10.00	8.295
10	1.9998	10.00	8.651
11	2.0013	10.00	9.587

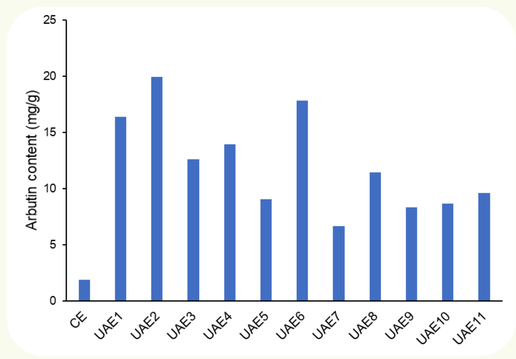


Figure 4. Arbutin content (mg/g) of extracts obtained by CE and UAE under different extraction conditions (UAE1-UAE11).

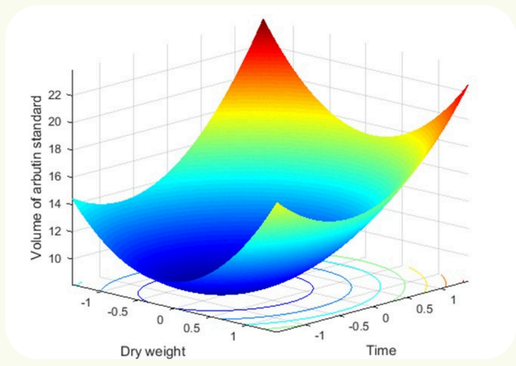


Figure 5. 3D response surface plots for Arbutin content (mg/g)
X1 = Dryweight and X2 = time.

Conclusion

- Ultrasonic-assisted extraction significantly improved the extraction efficiency of alpha-arbutin.
- The green extraction approach reduced solvent usage and extraction time.
- Analysis indicated that extraction time had a greater influence on the arbutin content than dry weight.
- Response Surface Methodology (RSM) successfully optimized the extraction parameters
- The optimal extraction parameters were 70% ethanol (v/v), 13.5 min, and 1 g, with RSM experimental design.

References

- Neural Concept. (2026). Response Surface Methodology – Overview & Applications. Retrieved from https://www.neuralconcept.com/post/response-surface-methodology-overview-applications
- MDPI. (2024). Extraction optimization of bioactive compounds using ultrasound-assisted extraction and response surface methodology. Horticulturae, 10(743). Lorem ipsum dolor sit amet, consectetur adipiscing elit. Mauris mauris lacus, tempor vel est quis, congue convallis sem. Nam vel

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