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A comparative study of sequential and simultaneous enzymatic and ultrasound-assisted aqueous two-phase extraction for anticholesterol compounds from *Strobilanthes crispus* leaves

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Abstract: An innovative ultrasonic-assisted enzymatic aqueous two-phase extraction (UAE-ATPE) method was applied to enhance the yield from *Strobilanthes crispus* leaves, exploring both sequential and simultaneous approaches. Comparative analysis included assessing total phenolic content (TPC), total flavonoid content (TFC), partition coefficient (k) and recovery (R). Liquid chromatography-mass spectrometry and scanning electron microscopy evaluated extracts from both techniques. Simultaneous UAE-ATPE demonstrated significantly higher TPC (5.7±0.1 mg GAE/g dry leaves) and TFC (3.3±0.1 mg QE/g dry leaves) compared to sequential extraction, where TPC and TFC measured 4.5±0.3 mg GAE/g dry leaves and 1.7±0.1 mg QE/g dry leaves. Additionally, simultaneous UAE-ATPE yielded higher k and R values for phenolic and flavonoid compounds. Notably, it identified 32.4% of the area corresponding to 6 compounds, surpassing the 25.3% area identified sequentially with 13 compounds. A collaborative effect of enzymatic hydrolysis and ultrasonic extraction was observed in simultaneous UAE-ATPE. In the inhibition test on the HMG-CoA reductase enzyme, simultaneous UAE-ATPE extract (200 µg/mL) exhibited exceptional results, achieving superior inhibition of 66.1% compared to the sequential method's inhibition of 39.4%. This underscores the efficacy of simultaneous UAE-ATPE in producing concentrated anti-cholesterol compounds. The study strongly emphasizes the superiority of simultaneous UAE-ATPE over the sequential approach.

Keywords: Enzymatic; Ultrasound; Aqueous two-phase; Extraction; Sequential: Simultaneous

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