

Title: Optimization of ultrasound-assisted extraction using response surface methodology for total anthocyanin content, total phenolic content, and antioxidant activities of Roselle (*Hibiscus sabdariffa* L.) calyces and comparison with conventional Soxhlet extraction

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Abstract: Response surface methodology (RSM) and a Box-Behnken design (BBD) were used to determine optimum conditions for ultrasound-assisted extraction (UAE) of *Hibiscus sabdariffa* L. calyces. The current study applied BBD to explore the effects of X1: ultrasonic temperature (30–80 °C), X2: ultrasonic time (20–50 min.), and X3: solid-to-solvent ratio (1:10–1:60) on total anthocyanin content (TAC), total phenolic content (TPC), and antioxidant activities (2,2-diphenylpicrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) assays). ANOVA results revealed that TAC, TPC, DPPH, and FRAP all had R² values of 0.98, 0.97, 0.98, and 0.98, respectively, indicating that models designed with second-order polynomials were capable of reliably analyzing interactions between parameters (response and independent variables) satisfactorily. It was determined from the RSM study that 80 °C ultrasound temperature, 48 min. ultrasound time, and 1:60 solid-to-solvent ratio were the optimum extraction parameters for maximizing TAC, TPC, DPPH, and FRAP. The experimental values for TAC, TPC, DPPH, and FRAP were 311 ± 5 mg CGE/100 g, 572 ± 7 mg GAE/100 g, 974 ± 3 µmolTE/100 mL, and 2332 ± 3 µmolTE/100 mL, respectively, under the optimal conditions. Also, a good agreement was found between experimental and predicted values, with a residual standard error of less than 5%. Compared to the yield of Soxhlet extraction for TAC (176 ± 4 mg CGE/100 g), TPC (210 ± 3 mg GAE/100 g), DPPH (534 ± 2 µmolTE/100 mL), and FRAP (1732 ± 3 µmolTE/100 mL), the extraction efficacy of the UAE process under optimized conditions demonstrated to be more effective. Therefore, based on the needs of the industry and sustainable development, the UAE process might be an economical alternative to traditional extraction methods.

Keywords: *Hibiscus sabdariffa* L., Calyces, Antioxidant activity, Response surface methodology, Ultrasound-assisted extraction, Soxhlet extraction

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