

Exploring the Effectiveness of Phenolic Antioxidants Extracted from *Annona muricata* L. Fruit Seeds and Peel Using Ultrasound-Assisted Extraction

A.A.K. Lankanayaka^{1,2}, K.G.L.R. Jayathunge¹, P.C. Bandara¹, D.C. Manatunga¹, S.M. Rajapaksha¹, C.M. Senanayake^{1*}

¹*Faculty of Technology, University of Sri Jayewardenepura, Pitipana, Homagama, 10200, Sri Lanka*

²*Faculty of Graduate Studies, University of Sri Jayewardenepura, Gangodawila, Nugegoda, 10250, Sri Lanka*

**chathurisnkn@sjp.ac.lk*

Utilizing antioxidants from fruit by-products promote sustainability in food industry by reducing waste. This study investigated the effectiveness of ultrasound-assisted extraction in extracting phenolic antioxidants from *Annona muricata* L. fruit seeds (ASE) and peel (APE), determining the total phenolic content (TPC), total flavonoid content (TFC), phytochemical composition, antioxidant activity, and antibacterial activity. Phenolic antioxidants were extracted using 100% ethyl acetate using both vortex-mixed and ultrasound-assisted extractions. TPC and TFC were determined using Folin-Ciocalteu's method and Aluminium Chloride colorimetric method respectively. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to identify the phytochemicals present in extracts. Antioxidant activity of the extracts was evaluated using ABTS⁺ radical scavenging assay and compared with synthetic antioxidants; butylated hydroxytoluene (BHT) and butylated hydroxyl anisole (BHA). Antibacterial activity against food-borne pathogens *Staphylococcus aureus* and *Salmonella typhi* was assessed by measuring the inhibition zone diameter via the disc diffusion method. Results indicated that ultrasound-assisted extracts resulted significant ($p < 0.05$) increase of TPC of ASE in 41% and APE in 48%, and TFC by 8% for both ASE and APE than vortex-mixed extracts. GC-MS analysis identified 1,2,3-Propanetriol, 9-Octadecenoic acid, n-Hexadecanoic acid in ASE and 1,2,3-Propanetriol, Oleic Acid, n-Hexadecanoic acid in APE as major phytochemicals. Half-maximal inhibitory concentration (IC₅₀ µg/mL) of ultrasound-assisted extracts of ASE and APE was 29±0 and 42±1 respectively compared to BHT (63±1) and BHA (97±1) while vortex-mixed extracts resulted 49±0, 55±0 respectively. Ultrasound-assisted extracts of ASE (9±1, 8±0mm) and APE (7±1, 7±0mm) showed higher inhibition against *Staphylococcus aureus* and *Salmonella typhi*. Respectively than vortex-mixed extracts of ASE and APE (8±0, 0±0 and 6±0, 6±0 mm respectively), while synthetic antioxidants showed zero inhibitory effect and therefore, they lack antibacterial activity. Ultrasound-assisted method is a potential extraction technique, and the tested natural sources could be promising alternatives to synthetic antioxidants due to their increased antioxidant and antibacterial activities.

Keywords: *Annona Muricata* L., Antimicrobial Activity, Antioxidant Activity, Phenolic Antioxidants, Ultrasonic-Assisted Extraction, Vortex-Mixed Extraction

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